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CONTENTS OF VOL LXIII

CONTENTS OF VOL LXIII

CONTENTS

LIST OF AUTHORS

PROCEEDINGS OF THE PHYSIOLOGICAL SOCIETY

LIST OF AUTHORS

OBSERVATIONS ON THE CONTRACTILITY OF LACTEALS Part II By HOWARD FLOREY

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THE appearances presented by the lacteals on microscopic examination of the mesentery in the guinea-pig and rat have been described in a previous communication(1) The rhythmical activities of these vessels have been examined, from the point of view of their initiation and control, both by means of nerve stimulation and drug action The appearances and reactions presented by the lacteals and lymph glands of other species are also described

It was possible with the microdissection apparatus to inject minute quantities of a drug close to the lacteal under observation, so that the effects of such an application could be seen on a circumscribed portion of the lacteal The pipette was first filled with the drug and the mesentery spread above the hollow chamber By suitable manipulation the drug was then injected and the results watched The drugs chosen were pilocarpine and adrenalin, as it was possible that the reactions obtained with them would give some indication of nervous influences on the rhythm Pituitrin was also used

In the rat, the injection of a small quantity of 1/100,000 adrenalin was followed by a vigorous contractile spasm in portions just near the injection and a much increased rate of contraction in portions a little further away The rate of contraction rose from once in 5 or 6 seconds to twice per second on occasion It must be admitted, however, that the reactions presented by these vessels were liable to be capricious, as also is the case in the cat, which will be described later This effect on the rhythm could not be produced in all experiments but, in that it occurred quite frequently, it must be accepted as significant The negative results may be attributable to some unknown factor, inflammation possibly, as it is known that blood vessels in a state of inflammation do not react by contraction to adrenalin In these preparations the blood vessels reacted invariably, but the lacteals seem to be possessed of a more delicate contractile mechanism

The same results have been obtained on the guinea-pig which was examined for this purpose in the modified apparatus to be described later A drop of 1/1000 adrenalin was placed on the lacteal by means of a fine glass rod This was rapidly diluted by the surrounding fluid, the degree of dilution being very considerable, but not accurately determinable In the case of the guinea-pig no spasm has been observed, but the rate is markedly increased, *e g* from a preliminary number of 8 times per minute to one of 14 per minute Portions of lacteal which were not contracting at the time of the application did not react in any way to the addition of adrenalin Lieben has also examined the action of adrenalin on the contractions of the lacteals of the rat(2) He finds that 1/20,000 adrenalin applied to the mesentery causes an increase in the activity of the vessels but if the drug be injected intravenously there is practically no effect

Pilocarpine This drug in strength of 1/10,000 had apparently no effect in the rat or the guinea-pig but when the strength was increased to 1/1000 complete inhibition usually occurred Use was made of this reaction to watch valve action under the high power of the microscope, as the movements were often sufficiently slow to be observed easily

Pituitrin This drug was capable of causing a spasmodic contraction occasionally but this result was quite infrequent

Nerve stimulation

The vagus had of necessity to be exposed in the neck While one observer stimulated, the other watched through the microscope In all cases there was a diminution in the amplitude of the contractions during the stimulus, a latent period of some 10 to 15 seconds intervening On the cessation of the stimulus there was a rapid return to the normal amplitude In some cases there was also a slight slowing but this was not frequent

Splanchnic The left splanchnic nerve was exposed through a large abdominal incision and cut The mesentery was arranged for microscopical observation Stimulation of the peripheral end of the splanchnic was followed in the majority of cases by an increase in the number of beats per minute, the rate in some experiments being doubled, *e g* from 12 beats to 24 per minute In another experiment from 3 to 6 beats per minute There was a considerable latent period from 15 to 30 seconds between the beginning of the stimulus and the increase in the rate *E g* a lacteal was beating at the rate of 3 per minute, a contraction at the beginning of timing being included During stimulation there was

a contraction at the beginning of timing, another after 30 seconds and two more during the second 30 seconds

On the cessation of stimulus the lacteal activity rapidly diminished and the count per minute then became less than before the application of the stimulus

However, that the seat of origin of the rhythmic contractions is in the vessel itself is indicated by the following experiments

The rat was chosen because of the greater ease in the manipulations, owing to the fact that the lacteals course further from the blood vessels in this species than in the guinea-pig A lacteal in which there was good rhythmic contraction was cut across by means of fine scissors This had no effect on the rhythm It was then cut across central to the previous section, there now being a completely isolated length of lacteal under observation. This isolated portion still contracted in a rhythmic manner The same result was obtained after isolation of the vessel by burning two points in its length with a fine hot needle A more conclusive demonstration that the rhythmic contractions were independent of the central nervous system was obtained by rapidly killing the animal (this experiment has been performed on both the rat and guinea-pig) by a blow on the head and mounting the completely excised mesentery and gut for observation in saline—the temperature being maintained at 37° C

Rhythmic contractions, normal in appearance, can be observed in these preparations for a few minutes after excision but they soon stop However, even after an hour a gentle stimulus with a rod is capable of initiating a complete beat at the point touched

It is suggested that the rhythmic action ceases relatively quickly because the normal slight dilatation (stretching) which precedes systole (see Part I) is not present owing to the cessation of the production of lymph It may be supposed that this stretch is essential for initiating contraction That a local mechanism is responsible for the contractions is also indicated by the fact that it was possible to observe in the intact mesentery, portions of lacteals contracting while adjacent portions were quiescent

These vessels in both rat and guinea-pig were very sensitive to mechanical stimuli, answering by a vigorous contraction, which remained quite local, and might be maintained for as long as 4 to 5 minutes If such a point of contraction were produced by bringing up a micro-needle quickly from beneath to touch the vessel, further evidence that the rhythmical contraction is a local phenomenon could be obtained The rhythms on the two sides of the point of contraction were quite different

in time relations and apparently unassociated That is, there was no
propagation of the disturbance past the strong contraction

The regular contractions have been observed to occur in these vessels
for $\frac{1}{2}$ to $\frac{3}{4}$ hour after the cessation of breathing, another fact which
would point to their peripheral origin

One may conclude therefore that the rhythm resides in the lacteal
wall itself, but that this may be modified by nervous influences Whether
this rhythm is initiated by ganglion cells or the contractile elements of
the wall is not decided, but histological investigations are in progress
to ascertain if the former are present

It was thought it would be of interest to examine other animals in
the same way to ascertain if the rhythmic contractions of the lacteals
was a universal phenomenon in mammals For this purpose the ap-
paratus was further modified so as to be applicable to animals of all
convenient sizes The trough and tray construction has been maintained,
these being made of the appropriate size for each type of animal Instead
of the hollow moist chamber, a glass cylinder, covered at the top with
a circular plate, was cemented over the hole cut in the bottom of the tray
The mesentery was spread over this and the gut fastened by pins to
cork cemented round the cylinder The whole apparatus could be filled
with saline so that the gut and the mesentery were immersed during the
whole time of observation A large electric light bulb was placed below
the tray to furnish transmitted light, and at the same time to aid in
keeping the saline warm Warm saline was also added from a reservoir
as occasion demanded A Zeiss binocular dissecting microscope was used
for these observations All animals were fed with fat so as to give the
maximum visibility to the lacteals The cat has been the animal for
study of the reactions in detail

In such a preparation of a cat no rhythmic movements such as are
seen in the rat and the guinea-pig could be observed though a large
number of animals have been examined, the greatest possible care being
taken to maintain them in good condition It was thought that the
anæsthetic (ether and cholorform, or urethane) might possibly be the
inhibiting agent Two methods to avoid this have been utilised A de-
cerebrate cat, from which most of the anæsthetic had been eliminated,
has been observed In this there were no rhythmic movements The
second involved the insertion of an abdominal window by a method
described elsewhere [3] A loop of gut was sutured round the cut edge of
the muscle so that a layer of mesentery was spread beneath the window,
being forced into apposition with the celluloid by pressure of the in-

testines behind After the animal had been allowed to recover for three days it was given a fatty meal When it was seen that the lacteals beneath the window were full of white fat, the cat was held by two assistants while the lacteals were observed by the low power of the microscope When the breathing was quiet it was possible to see the lacteals clearly In this case also no rhythmic contractions could be observed.

It may fairly be concluded that the cat does not possess these rhythmical movements as described in the other two species previously examined

The lacteals of the cat presented a smooth contour in the fully dilated condition, but when they were caused to assume a contracted condition by procedures to be described, they exhibited a sinuous outline, the valves being well marked by a bulging of the wall. In some cats there was a difference between the appearance of the lacteals of the duodenum and the upper ileum compared with those of the lower ileum The latter possessed more valves in their length, each being marked by a large bulge proximal to its site

The stream in these vessels could be observed by watching the ever changing opacity of the fat cloud in it For example, where two streams met, one of which was more highly charged with fat than the other, a separation of the two streams could be followed in exactly the same way as that described in the cerebral veins in similar conditions(4)

In the lacteals a continuous stream could be seen moving down to the lymph glands There did not appear to be any rhythmicity about the flow though the rate varied from time to time

If a vessel were punctured by a fine needle the portion central to the hole remained full, the contents being held up by the next proximal valve There was no evidence that this proximal portion emptied itself From the distal portion there was a continuous flow of lymph apparently independent of the peristalsis of the gut There was however evidence that the latter could alter the flow, for, in lacteals from a portion of the gut in active peristalsis, accelerations in the rate of flow were noticed when the gut was firmly contracted.

In certain suitable preparations it was possible to observe that at each inspiration the valves were opened and that in expiration they were shut again The influence of respiration could also be observed by examining the receptaculum chyli Eddies of fatty lymph synchronous with the respiratory movements could be seen where the clear lymph from the liver entered Under physiological conditions in the cat, there-

fore, it may be asserted that the forces moving the lymph are a *vis a tergo,* presumably supplied by the blood pressure, squeezing due to the gut peristalsis, and a respiratory suction pump mechanism The influence of respiration through increase in intra-abdominal pressure will be noted later There is no evidence that the lacteals, in a normal condition, further the passage of lymph by rhythmic contractions

Reactions to stimuli

Mechanical stimuli When these vessels were stimulated mechanically by means of a fine glass needle they underwent a considerable and rapid contraction, limited to the portion stimulated, in just the same way as that described for the cerebral arteries (4) This contraction could be of several minutes' duration but was never observed to be propagated

Electrical stimuli A contraction, which diminished the diameter of the vessel to one-quarter that present normally, could be produced by stimulation with a monopolar stigmatic electrode This contraction had the same appearance as that caused by mechanical stimuli

The reaction to these stimuli could in almost every case be evoked, but preparations were met with which were very refractory The reason for this is not apparent, as little difference between the preparations could be detected when the condition of these was judged from that of the circulation in the blood capillaries This fact of preparations occurring which did not react readily complicates the interpretation of negative results, but it was felt that if a certain result could be obtained at all then it was probable that it would be constantly present under strictly physiological conditions These remarks apply particularly to the action of drugs

Adrenalin Preparations have been made in which the lacteals react by vigorous contraction to a local application of adrenalin made by means of a glass rod or pipette There was no doubt that the contraction was due to the adrenalin, for if the saline were drained off the mesentery to free it from excess fluid, a single drop of adrenalin applied to a vessel produced a contraction only in the area to which the drop extended This experiment has been successful in several cases, but it must be admitted that vessels were frequently found which did not react in any way Camus, by the use of different methods, also concludes that adrenalin has a constrictive action (5)

Pilocarpine Pilocarpine has been applied in the same way as adrenalin This drug did not produce any appreciable effects on the calibre of the

vessels under any conditions Heinz states, however that pilocarpine causes contraction of the large lymphatic trunks(6)

Pituitrin This has also been applied in the same way In some cases there was a very small contraction, but in most experiments no result was obtained

Effects of nerve stimulation

Vagus The vagus was stimulated in the neck at the same time as microscopical observation was carried out No effects were seen to follow the stimulation of the nerves in the neck at any time though careful measurements were made with the ocular micrometer Paul Bert and Laffont(7) however, in working with dogs found that stimulation of the vagus caused a rapid and fugitive dilatation and then a contraction

Splanchnic This nerve was isolated in the right side just after its emergence from beneath the diaphragm the cat placed on its left side and the gut brought through a mid line incision. With such a rearrangement of the animal it was possible to stimulate the nerve and at the same time to observe microscopically Under these conditions a definite slight contraction of the lacteals was produced by stimulation of the splanchnic nerve In one experiment a diminution from 3 divisions of the micrometer scale to 2½ was observed. This contraction lasted several minutes In most of the experiments in which the splanchnic was stimulated, however, no contractions could be observed.

From the well marked effects with adrenalin and the less definite results from nerve stimulation it would seem probable that the vessels are innervated by the sympathetic system. Paul Bert and Laffont state that when the mesenteric nerves are stimulated the lacteals contract but that stimulation of the splanchnics causes dilatation Camus and Gley(8, 9) find that stimulation of the sympathetic nerves in most cases caused a dilatation of the cisterna chyli though in other cases a constriction was produced They also comment on the fact that it is sometimes difficult to elicit any reaction from these lymphatic structures

Experiments have therefore been made to investigate any possible reflex contractile mechanisms In asphyxia and stimulation of the central end of a sensory nerve the blood vessels undergo great contraction Accordingly, the trachea of a cat was exposed and clamped for a period during which the mesentery was examined The blood vessels were seen to contract but at no time was any contraction of the lacteals observed.

The same is true of observations carried out during the stimulation of the central end of the sciatic   One of the latter experiments was carried out in a decerebrate cat so as to have the reflex mechanisms of maximal sensitivity

It is usually considered that the blood is rapidly diluted in haemorrhage (Sherrington and Copeman(10), who quote Popp, *Ueber die Beschaffenheit des Blutes*, 1845, p 89, as the first to observe it), but recently Binet and Fournier(11) have stated that there is an initial increase in the number of red corpuscles owing to splenic contraction It was thought that the dilution which is generally held to occur might be brought about by the sudden emptying of the lymphatic system owing to the contraction of the walls of the lymphatics   Observations were made during rapid bleeding from a carotid canula   During the bleeding no contraction could be detected by the ocular micrometer   However, in one experiment, after the cessation of respiration, a very high grade of contraction appeared synchronously with vigorous gut movements That this was active contraction and not passive collapse was indicated by the fact that it was not possible to push the pocketed lymph into the contracted portions by means of a glass rod   One would also expect a vessel in passive collapse to present a regular smooth contour instead of these pockets which are similar to those which often appear in the mesenteric veins after death   Camus and Gley(12), however, report experiments in which they find that asphyxia causes a very marked contraction of the thoracic duct in a spinal animal, but that stimulation of a sensory nerve causes a reflex dilation of that structure

Several other animals have been examined microscopically in the same way

Man   During an operation on the intestine an opportunity occurred of observing the human lacteals[1]   The man was given 3 oz of olive oil 4 hours before the operation   At operation the lacteals were found to be full of chyle   Observation was carried out by means of a hand lens giving a magnification of 10   In this case there did not appear to be any rhythmical contractions comparable to those of the rat or guinea-pig   However, the lacteals were quite responsive to mechanical stimuli, a gentle tap with the gloved finger sufficing to cause a very marked local contraction at the point struck

It would seem that the human lacteals approximate to those of the cat in their properties, as judged from these necessarily limited manipulations

Squirrel (red)   A similar type of preparation to that of the cat was

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[1] I am indebted to Mr Perrin for this opportunity

made The mesentery was extremely transparent and, in places where there was no fat, a perfect view of the lacteals could be obtained In this animal also no peristaltic contractions could be seen The lacteals were contractile to mechanical stimuli right up to the point where they emerged from the gut wall, a fact of some importance when considering the structure of these vessels No contraction was observed to take place on the application of adrenalin but it was found that the vessels then contracted very much more readily to mechanical stimuli than before the application

Rabbit This animal offered considerable difficulty in observation owing to the very vigorous continuous peristalsis of the intestine This imparted movements to the whole of the mesentery with a consequent passive distortion of the contained lacteals No active rhythmic contractions have at any time been observed These vessels, however, possess the power to contract to mechanical stimuli in the same way as those of the other species examined No effects from the application of adrenalin were seen Muller and Schwann(13) did not observe any rhythmical contractions of the lacteals of this animal

Dog A puppy has been examined in the same way but does not possess the property of rhythmical contraction of its lacteals Here also the vessels were contractile to mechanical stimuli

Hedgehog This animal readily took cream, so the lacteals showed up extremely well. The vessels of this animal were far more abundant than those of any of the other species examined They were richly anastomotic, frequently forming closed loops The vessels often divided in an apparently purposeless fashion, to be reunited a short distance away The meaning of these rings is not clear

No spontaneous contractions were observed, though the preparation, as judged by the state of the blood circulation, was excellent

The vessels, for the most part, presented a smooth contour, but they were bulbous in places not apparently associated with valves The addition of adrenalin made the wall take on a finely wavy outline as if the lacteal outline had been traced by a tremulous hand It was thought that this appearance was caused by the contraction of the very few muscle fibres possibly contained in these vessels Mechanical stimuli were effective but these structures were not very contractile As they were easily deformed passively, care had to be taken to distinguish the active contraction

Mouse Reflected light from a 100 candle-power Pointolite lamp was found to give a very beautiful picture in this animal

The mesenteric lacteals were very fine and presented numerous bulbous swellings

The gut in this animal was very transparent and it was possible to see two plexuses through its walls, one, just beneath the muscle, possessing coarse irregular meshes with numerous bulbous swellings, and a deeper one (presumably submucous) with fine regularly spaced meshes with very few swellings This appearance was especially well seen after the peristalsis of the gut had been inhibited by the addition of adrenalin

The influence of the peristalsis of the gut on the propulsion of the chyle was beautifully seen in this preparation Each movement was observed to compress the lacteals contained in the gut wall and as these are furnished with valves the contained fluid was forced along

If the gut was stimulated by a fine needle it contracted at the point of stimulus sufficiently to free the area stimulated so completely of the lymph that the vessels disappeared from view, to return as the spasm of the gut wall passed away The lacteals in the mesentery were not rhythmically pulsatile Mechanical stimuli produced only a slight contraction

Adrenalin did not cause any evident contraction Portions of the lacteals of considerable length appeared to be in a contracted condition throughout the observation but these portions did not vary in calibre It was impossible to say whether these portions were actually contracted or whether the appearance was due to the structure of the lacteal

Lieben(2), however, states that he observed rhythmical contractions in the mouse, but considered those seen in the rat much better The present observations do not confirm his statement with regard to the rhythmicity The mouse lacteal is also very different in its susceptibility to mechanical stimuli from that of the two species presenting rhythmical contractions In the latter, one touch produces extreme contraction, in the former, vigorous stroking only produces the slightest effects

Pig A young sucking pig about five weeks old was used for these observations Though the animal possessed voluminous intestines, the lacteals from which were adequately filled with fat, it was very difficult to observe the mesentery owing to its extreme shortness making a withdrawal of it from the body cavity awkward Reflected light was used for the examination owing to the impossibility of stretching the mesentery over the glass cylinder No rhythmic contractions were observed With vigorous mechanical stimuli it was possible to cause a localised contraction

—but this was difficult to elicit Pituitrin was not observed to have any effect Adrenalin also caused a slight contraction in some places but this effect was minimal

An interesting phenomenon has been observed in many of these animals, so many as to suggest that it is common to all species As has been described in the guinea-pig, after the gut and mesentery have been exposed for some time, red corpuscles together with polymorphs appear in the lymph stream The polymorphs are recognised in specimens fixed and stained The red corpuscles may be so numerous as to give the lacteal an appearance like a vein in which the current is very slow In some animals, where pins have been put through the gut to steady the preparation, the red cells appear very quickly but gross trauma of this kind is not necessary for their occurrence It would seem that a very moderate degree of inflammation is capable of allowing the diapedesis of the red corpuscles from the blood vessels and at the same time allowing the lacteals to take up these cells

It is now held that the lymphatic system is a closed one which means that the lacteal walls must have been altered in their permeability to allow particles of the size of red corpuscles to pass An extension of these observations might throw some light on the mechanism by which the lacteal takes up fat and, in general, bodies of particulate matter The observations would indicate at least that during an inflammation the lacteals also alter their permeability This observation is in line with the fact that some bacteria are absorbed through the lymphatics

Of the series of animals thus examined only the rat and guinea-pig present the phenomenon of rhythmically contractile lymphatics It was thought that possibly the venous pressure in these animals was very high necessitating the intervention of active pumping to force the lymph into the venous system where in other animals the accessory factors—respiration, etc —were sufficient for this purpose However, this is apparently not the explanation

A guinea-pig anæsthetised with urethane was found to have an arterial pressure of 85 mm Hg, as measured in the left carotid artery, with a corresponding venous pressure, taken in the peripheral end of the jugular vein, of 9 cm of sodium citrate solution The ratio of the venous to arterial pressures would appear to be of the same order as that usually present in mammals

Another method has been used for investigating the properties of these structures in the cat

From the glands at the base of the mesentery one large and one

or more smaller ducts emerge The large duct is usually about the size of the jugular vein In a fat fed animal the duct was seen easily when approached through a large ⊥-shaped incision in the left side The spleen and stomach were pulled over to the right The duct is very delicate and great care had to be exercised in clearing it Only sufficient was cleared for the passage of a ligature as it was found advantageous to retain the connective tissue in the region of the incision made to receive the canula, otherwise the duct collapsed to a thread and the insertion of the canula became a very tedious and sometimes impossible task Before the insertion of the canula, in some experiments, all the nerves accompanying the superior mesenteric artery were ligated and severed After the canula was tied in, it was held firmly in a specially constructed small canula holder, possessed of a universal joint movable on a long horizontal arm, the whole mounted on a vertical pillar attached to a heavy base The canula was then connected either to a fine tube for drop counting or to a vertical manometer This preparation was first made in the hope that some light might be thrown on the behaviour of lymphatic glands to stimulation of the nerves It was however apparent that in such a system one also had to take into account the preglandular lymphatics, as pressure on a loop of gut could expel lymph from the canula (an observation made long ago by Heidenhain) It was not possible to block the preglandular area without depriving the glands of lymph

There was one serious source of error to be ruled out in making experiments in which drops were counted The slightest pressure from the finger on the glands during any manipulations was capable of forcing out lymph and so adding to the drop flow In the same way the influence of respiration was surprisingly large The abdomen was freely opened and there could be no question of a suction pump action of the thorax and yet in many experiments alterations in the rate and depth of respirations were capable of causing an increased outflow In the experiments to be recorded great care was taken to exclude these factors Evidence of the exclusion of the respiratory factor was obtained by stimulating the central end of the saphenous nerve at the close of observation and finding no influence on the rate of outflow

By this method also several experiments were made in which nerve stimulation gave no result though care was taken to keep the mesentery and gut warm by saline pads and frequent irrigation with warm saline The figures obtained in experiments exhibiting a reaction on stimulation of the mesenteric nerves are appended

Exp 1 *Stimulation of mesenteric nerves*

    1 drop in 2 minutes before stimulation.
    3 drops ¼ minute during stimulation.
    1 ,, 1 ,, with no stimulation.
    2 ,, 1 ,, with stimulation.

Exp 2 *Stimulation of mesenteric nerves*

    1 drop in 1 minute before stimulation.
    8 drops 1 ,, during stimulation
    3± ,, 1 ,, during minute after stimulation.
    Interval of 10 minutes.
    0 drop in 1 minute before stimulation.
    4 drops 1 ,, during stimulation.

Exp 3 *Stimulation of mesenteric nerves*

    4± drops in 1 minute before stimulation
    6± , 1 ,, during stimulation.
    3— , 1 ,, after stimulation
    Repeated with the same numerical result

From these figures it will be seen that there is a definite action on the amount of lymph coming from the canula during stimulation of the nerves to the glands

Measurements on lymphatic pressure have also been made   In a similar preparation a vertical saline manometer was connected by a rubber tube to the canula and the height to which the column rose noted A correction was made on account of the capillarity of the tube The level rose slowly from zero—each respiration causing an oscillation of about 2 mm   In most experiments the pressure measured was about 7 5 cm with a contemporaneous venous pressure—measured in the central end of the splenic vein—of 11 cm of saline Owing to the lymphatics being a valved system, if saline was poured into the manometer the level was maintained at that produced by the addition of the fluid It was thought that when the manometer level had risen from zero to a resting state— of about 7 5 cm —stimulation of the mesenteric nerves should give a further rise if this procedure caused a contraction of the lacteal walls and of the glands However, in several experiments this expectation was not realised.

In one experiment in which 1 c c of adrenalin (1/10,000) was introduced into the saphenous vein no alteration in pressure was observed

The effects of stimulation of the sciatic and hæmorrhage were tried, but beyond raising the pressure 2–3 mm , probably owing to the increased respiration, there was no result

The injection of pilocarpine however produced a striking result

1–2 c c of 1/1000 solution was injected into the saphenous vein Within 15–20 seconds the pressure in the manometer began to rise rapidly from 7 2 to 14 5 cm Having reached the highest level it was of course maintained there, but if after an interval of a few minutes the level were lowered by sucking the fluid from the manometer the pressure was then found to have declined As is well known, pilocarpine acts as a lympha gogue and in these experiments the canula, which before the injection had filled slowly with lymph, now filled rapidly This was observed to be the case during a disconnection of the manometer for the removal of possible clot The amount of extra fluid rapidly forced into the mano meter was of course lymph That, however, this amount of extra lymph was not caused by a sudden contraction of the lacteals and glands was shown by the fact that when the canula was disconnected from the manometer the greatly increased flow continued for several minutes If the contraction of some muscle in the lacteals and glands had been the cause one would have expected one gush of lymph and then no more The explanation of this considerable rise of pressure would seem to lie in the greatly increased peristalsis of the gut In some places the gut was at times so contracted as to appear white That gut contraction is a potent factor in forcing lymph onwards has already been indicated The increased respiratory movements may also have contributed to the rise in pressure The other factor allowing the peristalsis to give its maximum effect, is the increase in the amount of lymph produced

An attempt to test the truth of this explanation was made by injecting pituitrin which also causes increased gut movements In these experiments a trifling rise of 1–2 cm was produced However, the gut peristalsis was at no time as vigorous as that produced by pilocarpine At the same time pituitrin produced extreme contraction of the vessels and consequently a greatly diminished lymph flow so that the effects of the two drugs are in no way comparable

To obviate the necessity of considering the contractility both of the glands and the lacteals in experiments of this nature, some were performed on isolated glands These glands were taken from the mesenteries of cats which were first chloroformed and then killed by a blow on the head The glands were then dissected free from fat and peritoneum, being touched as little as possible, and then immersed in cold Ringer Locke till required A suitable gland was then suspended in oxygenated Ringer Locke at 37° C and attached to a light lever which made a tracing on a drum In the tracings appended one can see that the addition of adrenalin caused a perfectly definite contraction This was

repeated on three glands of the same cat with similar results There was also a well marked contraction when barium chloride was added The addition of pilocarpine produced no contraction, nor did histamine, in these preparations, appear to have any action As in all the present experiments on the motor activities of the lacteals, this result could not be obtained invariably, preparations were met with which were quite inert
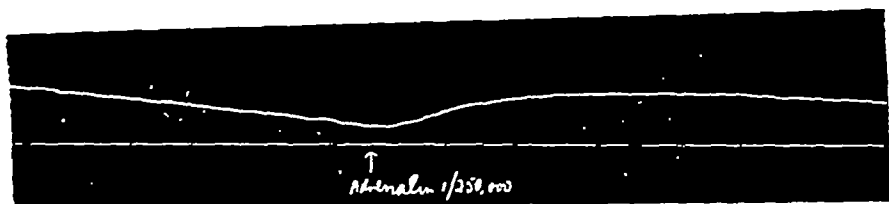
Fig 1 Isolated lymphatic gland. Cat Contraction produced by addition of adrenalin.
Lever magnification 16
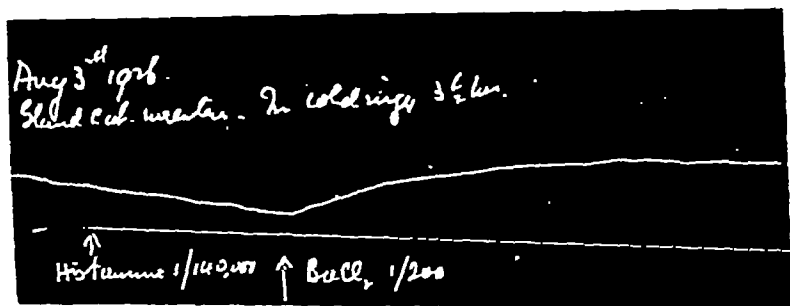
Fig 2 Isolated lymphatic gland. Cat Contraction produced by addition of BaCl₂
Lever magnification 16

It might be objected that the results obtained in these experiments were due to the contraction of the smooth muscle of the contained blood vessels To meet this possible objection similar preparations of pancreas, liver, kidney and parotid salivary gland were made In these preparations the addition of the drugs were without effect except in the case of the liver, in which a slowly falling curve was straightened out In all of these structures there is at least as plentiful a supply of blood vessels as in the lymph glands That even in the lymphatic gland the result cannot always be obtained is another argument against the result being attributable to the contained blood vessels Blood vessels are

very resistant and will invariably react to adrenalin under such conditions

Harvey(14) is of the opinion that pilocarpine causes contractions of the lymph glands as well as the spleen, as judged from counts of lymphocytes circulating in the blood   Peyton Rous(15) noted that struggling and movement increased the output of lymphocytes from the thoracic duct, a fact which could be explained by pressure on the lymphatic glands   He was also inclined to the view that pilocarpine causes contraction of lymph glands though he observes that an injection of the drug is capable of making a clear lymph chylous for a short time   Gerlach noticed muscle fibres in glands and that they contracted on electrical stimulation   This statement is taken from Heller's paper as, unfortunately, Gerlach's original thesis is not available

According to Emminghaus(16), Malpighi suggested that the muscular tissue of lymph glands contracted and forced on the lymph, while Boerhaave suggested that rhythmical contractions of the thoracic duct might be a factor in propelling the lymph

With the foregoing evidence that the lymphatic glands are contractile, even if only slightly so, a series of counts was made of the corpuscular contents of the lymph issuing into a canula inserted in the manner before described   As the canula was inserted close to the gland one could be reasonably certain of obtaining a correct index of the lymph flowing from the gland or glands before any admixture with other lymph or sedimentation of the corpuscles had occurred   In the following experiments the mesenteric nerves were stimulated   The procedure was to allow the lymph to collect in the canula   This was then rapidly collected in a pipette and transferred to a watch glass, from which the counts were made   The manipulations had to be carried out expeditiously as it was found that the lymph clotted very quickly

Exp 1   Canula inserted and lymph immediately collected   Count 1   14,320 per c mm   15 minute interval

Count 2   21,400 per c mm   Interval of 2 minutes

Count 3   18 200 per c mm.

Stimulation of nerve for 60 seconds.

Count 4   88 000 per c mm.

The vast majority of the cellular elements present were lymphocytes together with a small number of mononuclear type of cell

Exp 2   Count made immediately after insertion of canula.   8600 per c mm   15 minute interval   Count 1400 per c mm

Stimulation for 1 minute   6400 per c mm

Exp 3   Lymph expressed into canula accidentally after insertion of canula.
Count 1 65,000 per c.mm.
15 minute interval with a good flow during the period.
Count 2 36,875 per c.mm.
Stimulation of nerves along the vessel.
Count 3 43,100 per c.mm

Exp 4. 10 minutes after the insertion of the canula the first count was made. There had been a good flow of lymph during that period.
Count 1 8900 per c.mm.
Stimulation for 1 minute of the mesenteric nerves
Count 2 14,250 per c.mm

In these experiments the same care was taken to avoid mechanical interference as in the dropping experiments In Exp 3 evidence was obtained that slight pressure, such as from a finger lightly applied, was capable of causing a great increase in the number of cells expelled In Exp 4, after the first two specimens had been collected, light pressure was applied to the gland and the lymph so expressed counted Count 3, 21,000

The effects of drugs have also been studied In one such experiment the initial count was 11,600   25 c c of pilocarpine was then given intravenously The count made on lymph collected 2–3 minutes after was 17,000 After the animal had apparently returned to normal 5 c c of 1/10,000 adrenalin was injected and a count then made of 25,000

From this it would seem that adrenalin has a greater power to increase the concentration of lymphocytes issuing from the gland than pilocarpine, an observation which would fall into line with those already made on the contractility of the glands to adrenalin

It will be seen that Exp 1 seems to indicate that the stimulation causes an expression of lymphocytes from the gland. In Exp 2 the relatively high initial count may be due to the unavoidable mechanical interference during the insertion of the canula There is a marked rise after the stimulation, although the absolute figure is not as great as that counted at the beginning of the experiment In this experiment all counts were low compared with the others Exp 3 gave a result in which there was a move in the direction of increase but the difference is such as to be negligible

In Exp 4 however there is a very definite increase in the number of cells present after the stimulation This experiment also demonstrates the very great influence of pressure on the expulsion of the cells from the glands

SUMMARY

1   The rhythmical contractile lacteals of the guinea-pig and rat are under the control of the nervous system, though the rhythmicity is of peripheral origin

2   The lacteals of the cat, dog, rabbit, squirrel, hedgehog, mouse, pig and man, do not exhibit rhythmical contractility similar to that of the guinea-pig and rat

3   The lacteals of these species are however contractile, exhibiting this property especially to mechanical stimuli.

4   The lacteals of the cat, which have been specially studied, contract to adrenalin and sympathetic stimulation

5   The influence of respiration and gut peristalsis on lymph flow is well seen in these preparations

6   Lymphatic glands contract when stimulated by adrenalin but not with pilocarpine

7   There is an increase in the number of lymphocytes issuing from the mesenteric lymph glands when the mesenteric nerves are stimulated

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THE SYNERGISM BETWEEN ALCOHOLS AND OTHER DRUGS By L M PICKFORD

(From the Pharmacology Department, University College, London)

THE chief object of this research was to determine whether an antagonism of the type observed by Warburg(1, 2) between cyanides and urethanes could be demonstrated on the isolated frog's heart between other drugs

Warburg measured the oxygen consumption of bird s red blood corpuscles and sea urchin's eggs and found that although this was decreased by both urethanes and cyanides, yet the presence of urethane decreased the amount of action produced by cyanides In a later paper (3) he has developed the thesis that narcotics inhibit respiration by being adsorbed over the cell surface whilst cyanides produce specific action by uniting with and inactivating iron, and that narcotics interfere with the action of cyanides by occupying the receptors on which this drug acts

Technique The preparation studied was that described by Clark (4), namely, a strip of frog s ventricle driven with an artificial rhythm. The strip was suspended between electrodes in a small bath through which a steady flow of fluid was passed from an inlet above to an outlet in the floor of the chamber This obviated any errors due to evaporation or accumulation of metabolites As the bath held only 5 c c of fluid and the inlet and outlet tubes were comparatively large, emptying and refilling could be carried out in a very short time Movements of the strip were recorded with an isometric lever

The chief source of error observed was the dependence of the activity of the strip on the force with which it was washed by the perfusing fluid. The frog s ventricle is a sponge-like substance, and, if suspended in a solution. products of metabolism accumulate and the force of contraction gradually diminishes This error was avoided either, as mentioned above, by allowing a steady stream of fluid to flow over the strip. or, where this was impracticable, by washing out the strip frequently by means of a pipette with the solution that was being tested. Another source of error was the change in sensitivity of the strip This variation was observed both between different hearts and between the responses of the same heart

during the course of an experiment This was noted by Vernon(5) in the case of chloroform As regards variation between different hearts it was found after a number of experiments that the large majority gave a response fairly close to the mean value, therefore extreme cases have been discarded As regards the variation in response of the same heart at different times, it was found that when first isolated hearts were relatively insensitive to alcohol and that the sensitivity increased later, but that after isolation for an hour or two the sensitivity became fairly constant

Action of alcohols on the heart Experiments were first made to determine the relation between concentration and the amount of action produced by alcohols It was observed that with fresh hearts, the heart showed a power of partial recovery, and the initial depression produced was greater than in the final equilibrium (Wind(6) quotes Haffner as observing a similar recovery effect in the frog's heart with alcohol and other drugs) After isolation for an hour or two the partial recovery was absent, but the depression produced was found to be the same as that produced initially in the fresh heart These errors make it difficult to observe exactly the relation between concentration of alcohols and action in the frog's heart

Previous observers have noted both curved and linear relationships Linear relationships were described by Warburg(1) (urethanes on the oxygen consumption of bird's red blood corpuscles), Vernon(5) (alcohols on the isotonic record of the isolated tortoise heart), Storm van Leeuwen and le Heux(7) (urethane on reflexes of decerebrate rabbit) A curved relationship was found by Meyerhof(8) when studying the action of narcotics on the respiration of denitrifying bacteria, for when the concentration was increased the action produced increased at more than linear rate Shackell(9) found that the destruction of *Limnoria* by ethyl alcohol followed a similar curve, and attributed it to a frequency effect

In the present experiments methyl, ethyl, *n*-butyl and *n*-octyl alcohols were studied Each of the following curves is taken from a single experiment in order to show the variation in response of one heart to the same concentration of narcotic, the curves are, however, typical of the average responses of a large number of hearts It can be seen in Fig 1, Curve I for ethyl alcohol, Curve II for methyl alcohol and Fig 2, Curve IV for butyl alcohol, that the relation between concentration and action was nearly linear when actions less than 80 p c of full inhibition were produced The deviation from the linear relation was almost within

the limits of experimental error, and Fig 2, Curve II shows a case where
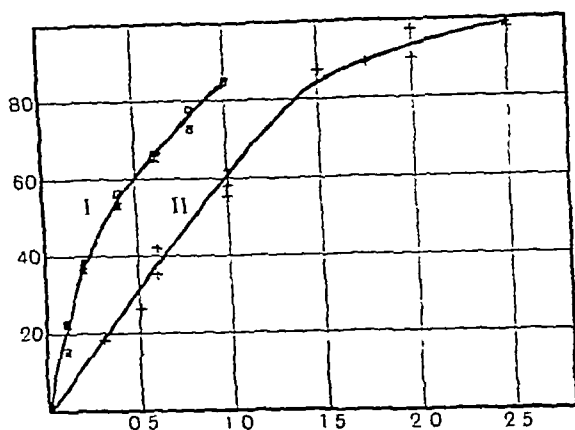
Fig. 1   Action of alcohols on the contraction of the frog s heart. Ordinate per cent. reduction of contraction. Abscissa molar concentration. Curve I. Ethyl alcohol. Curve II. Methyl alcohol.

a linear relation was observed   The results, therefore, are not conclusive but suggest that a curved relation is the more probable   The non-linear relation found resembles that shown by Meyerhof(10) for the relation between concentration of alcohols and the inhibition of invertase

The depression of contraction under the action of alcohol was very rapid, as also the recovery   Concentrations sufficient to produce a 50–20 p c reduction in beat produced half action in from 1 5 to 5 seconds, and half recovery occurred in about 5 seconds

The relative activity of the different alcohols was not measured very accurately, but from curves of average values a 50 p c reduction in hearts after a few hours' isolation was produced by the following concentrations
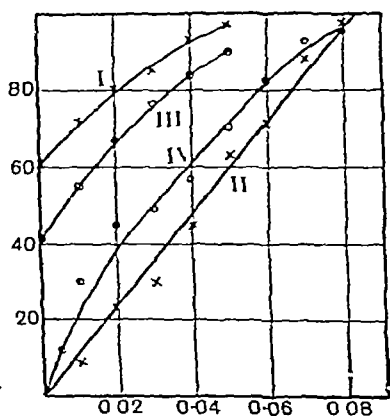
Fig 2   Action of butyl and methyl alcohols on frog s heart   Isometric records of heart strip   Ordinate and abscissa as in Fig 1

Curve I. 1·0 molar methyl alcohol plus varying concentrations of butyl alcohol

Curve II   n butyl alcohol alone

Curve III. 0 6 molar methyl alcohol plus varying concentrations of n butyl alcohol

Curve IV   n-butyl alcohol alone

The records were taken from one heart in the order indicated above

Methyl alcohol	0 9 molar
Ethyl alcohol	0 35 molar
n-Butyl alcohol	0 03 molar
n-Octyl alcohol	0 0003 molar

These results follow the general rule that in the series of normal alcohols when the length of the chain is increased by one carbon atom the intensity of physiological action is increased about three-fold This rule has been found to hold for a very large variety of physiological actions (Winterstein[11])

Synergism of alcohols A very large amount of work has been done to determine whether, when aliphatic narcotics are mixed, the physiological action of the mixture represents a simple addition of the action of the components or whether potentiation occurs The great majority of writers agree that a simple addition occurs In particular the following writers found a simple additive effect when alcohols were mixed Fuhner[12] (hæmolysis), Grilichess[13] (narcosis of rabbits), Kissa[14] (paralysis of *Colpidium*) Kissa indeed found that mixtures of alcohols had less effect than the sum of the separate actions and a similar conclusion was reached by Bills[15] who studied the paralysis of *Paramœcium* Warburg[1] found that the action of mixtures of urethanes in reducing the oxygen consumption of bird's red blood corpuscles was a purely additive effect

The effect of the isometric response of heart strips of combining alcohols is shown in Fig 2 It can be seen that for concentrations giving less than 80 p c inhibition the effects are additive, since Curves I and II and Curves III and IV are nearly parallel

With low concentrations the effects produced by combinations of alcohols might be interpreted as a simple additive action With higher concentrations, however, the combination of two alcohols produces much less action than the sum of the separate actions These results could be considered additive if allowance be made for the fact that the relation between the action and concentration of alcohol is a curved and not a linear relation For obviously if this relation between concentration and action follow anything other than a straight line, the effect of adding two alcohols A and B will not equal the addition of the separate actions, but the anticipated action can be calculated easily from the curves relating the concentration and action of either of the two components For example, if alcohols A and B are present in concentrations a and b respectively, and if B in concentration b produces the same action as A in concentration $a/5$, then the combined action will be equal to that of A

in concentration $a + a/5$ A series of results with octyl alcohol is shown in Table I   The values given are the averages of a large number of experiments   It can be seen that the calculated values (in brackets) approximate very nearly to those observed

<div align="center">TABLE I</div>

Per cent inhibition produced by ethyl and octyl alcohols alone and combined

Molar conc. ethyl alcohol		0	0 5	1 0
Molar conc. n octyl alcohol 0		—	32	60
	0 0004	36	59 (64)	80 (82)
	0 0006	50	69 (72)	85 (88)
	0 0008	67	81 (82)	92 (96)

Butyl chloral hydrate   Butyl chloral hydrate was chosen as an example of a powerful narcotic   Fig 3 shows that 0 0015 molar solution produces a 50 p c inhibition of the heart, and that the relation between concentration and action is somewhat similar to that of the alcohols   As in the case of the alcohols the heart when first isolated was much less susceptible than after prolonged isolation   The action of butyl chloral hydrate is, however, of a different character from that of the alcohols because the time relations are totally different   The time for half action and half wash out in the case of the alcohols is less than 5 seconds, whereas butyl chloral hydrate acts nearly one hundred times more slowly, as is shown by the figures in Table II

Fuhner[12] noted that the rate of action of chloral hydrate in producing hæmolysis was much slower than that of the alcohols

<div align="center">TABLE II.</div>

Molar concentration of butyl chloral hydrate	0 001	0 004	0 01
Time in seconds required for half action	480	180	80
Time in seconds required for half recovery on washing out	360	400	480

Synergism of alcohols and butyl chloral hydrate   The action of mixtures of methyl alcohol and butyl chloral hydrate is shown in Fig 3   It will be seen that 0 6 molar methyl alcohol produced the same action as 0 0012 molar butyl chloral hydrate, but the effect produced by methyl alcohol and butyl chloral hydrate acting together was considerably less than can be accounted for by supposing the methyl alcohol to act as if an extra 0 0012 molar butyl chloral hydrate were present   At the same time the inhibition produced by any given mixture of the two drugs is always greater than that produced by either of the two constituents acting alone   The same is true for the experiments with 1 0 molar ethyl alcohol and

butyl chloral hydrate Therefore it can scarcely be said that there is
an antagonism between the two drugs, interference seems a preferable
term

The interference observed can be
explained on Warburg's hypothesis that
the alcohol covers the surface of the
cell and that the other drug cannot
react with that portion of the cell covered
by the alcohol Therefore the higher
the concentration of alcohol the smaller
will be the free surface upon which the
butyl chloral hydrate can exert its effect

Owing to the slow rate of action and
wash out of butyl chloral hydrate it is
particularly difficult to get a series of
accurate observations with this drug,
and the measurements are not suf-
ficiently accurate to bear mathematical
analysis

As previously mentioned, butyl
chloral hydrate reacts with, and is
removed from the heart on washing out

Fig 3   Action of butyl chloral hydrate
and alcohols on frog's heart. Ordin
ate and abscissa as in Fig 1

Curve I   Butyl chloral hydrate alone
Curve II   0 6 molar methyl alcohol
plus varying concentrations of butyl
chloral hydrate
Curve III   1 0 molar ethyl alcohol
plus varying concentrations of butyl
chloral hydrate

nearly one hundred times more slowly than alcohol This difference in
time relations is very well shown by replacing a mixture of the two
drugs by butyl chloral hydrate alone in unaltered concentration, the
removal of the alcohol leaves uncovered a considerable portion of the
surface of the heart cells, thus there is an immediate partial recovery,
followed by a gradual depression as the butyl chloral hydrate slowly
reacts with the new surface left exposed

Action of cyanides Warburg[1] found a linear relation between
concentration of cyanides and their action in depressing the oxygen
consumption of bird's red blood corpuscles Gray[16], however, found
a logarithmic relation between the concentration of cyanides and their
action, in depressing both the oxygen consumption, and also the activity
of the ciliated epithelium of *Mytilus edulis* Vernon[5] also observed
that the relation between concentration of cyanides and depression of
the isotonic contraction of the tortoise ventricle was not a linear relation

I found that the action of cyanides was profoundly influenced by
the condition of the heart, for a fresh heart was often ten times as re-
sistant as a partly exhausted heart, but after a few hours the response

of the heart became fairly uniform When partly exhausted hearts were studied the relation between concentration and action was certainly not a linear one and appeared to approximate to a hyperbola resembling that described by Clark(4) in the case of acetyl choline The dotted curve in Fig 4 is drawn to the formula $Kx = \frac{y}{100-y}$ where $x =$ molar concentration, $y =$ percentage inhibition produced, and $K =$ constant $= 5750$, it will be seen that it fits the observed figures fairly well. (The abscissa $= \log$ of molar concentration for the sake of compression.)

Cyanides act considerably slower than alcohols, and the times for half action and half recovery on washing out he between 50 and 100 seconds for concentrations between 0 01 and 0 0001 molar cyanide

Synergism of cyanides and alcohols Warburg(1) found that alcohols actually antagonised the

Fig 4. Action of sodium cyanide on frog's heart. The broken line is calculated from a formula given in the text. Ordinate per cent. reduction in contraction. Abscissa log of molar concentration. The observed values are the averages of a number of experiments

effect of cyanides in diminishing the oxygen consumption of sea urchin's eggs I found no antagonism between cyanides and alcohols but a combination of actions similar to that observed with mixtures of butyl chloral hydrate and alcohols The alcohol appeared to cover a portion of the heart surface and the cyanide to act on the remaining surface

Table III shows the results obtained in a series of experiments of combining alcohol and cyanide. It can be seen that the presence of alcohol causes a considerable interference with the action of cyanides, but that a mixture of the two drugs always produces a greater action than either of the constituents alone, in the same concentration as they occur in the mixture Between each determination of the effect of combinations the response to the single drug was observed in order to allow for any increase in sensitivity during the course of the experiment, this was necessary as unfortunately the sensitivity is changed by repeated application of cyanide, and therefore it is difficult to obtain

butyl chloral hydrate Therefore it can scarcely be said that there is an antagonism between the two drugs, interference seems a preferable term

The interference observed can be explained on Warburg's hypothesis that the alcohol covers the surface of the cell and that the other drug cannot react with that portion of the cell covered by the alcohol Therefore the higher the concentration of alcohol the smaller will be the free surface upon which the butyl chloral hydrate can exert its effect

Owing to the slow rate of action and wash out of butyl chloral hydrate it is particularly difficult to get a series of accurate observations with this drug, and the measurements are not sufficiently accurate to bear mathematical analysis

As previously mentioned, butyl chloral hydrate reacts with, and is removed from the heart on washing out

Fig 3   Action of butyl chloral hydrate and alcohols on frog's heart   Ordinate and abscissa as in Fig 1

Curve I   Butyl chloral hydrate alone.
Curve II.   0 6 molar methyl alcohol plus varying concentrations of butyl chloral hydrate
Curve III.   1 0 molar ethyl alcohol plus varying concentrations of butyl chloral hydrate

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I found that the action of cyanides was profoundly influenced by the condition of the heart, for a fresh heart was often ten times as resistant as a partly exhausted heart, but after a few hours the response

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Cyanides act considerably slower than alcohols, and the times for half action and half recovery on washing out lie between 50 and 100 seconds for concentrations between 0 01 and 0 0001 molar cyanide

Fig. 4. Action of sodium cyanide on frog's heart. The broken line is calculated from a formula given in the text. Ordinate per cent. reduction in contraction. Abscissa log of molar concentration. The observed values are the averages of a number of experiments

Synergism of cyanides and alcohols Warburg[1] found that alcohols actually antagonised the effect of cyanides in diminishing the oxygen consumption of sea urchin's eggs I found no antagonism between cyanides and alcohols but a combination of actions similar to that observed with mixtures of butyl chloral hydrate and alcohols The alcohol appeared to cover a portion of the heart surface and the cyanide to act on the remaining surface

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a large number of comparable results which can be plotted in a series of curves

TABLE III. Action of sodium cyanide and ethyl alcohol alone and combined on the frog's heart

I Sodium cyanide									
Molar conc	0 0003	0 0003	0 0003	0 001	0 001	0 001	0 003	0 003	0·003
Per cent inhibition produced	21	15	47	80	80	74	79	80	94
II Ethyl alcohol									
Molar conc	0 2	0 4	0 8	0 4	0 6	0 8	0 1	0 2	0 6
Per cent inhibition produced	38	52	71	53	67	78	22	37	65
III. Combination of I and II									
Per cent inhib	47	59	84	90	87	94	84	86	99 4

Warburg[1] found that the inhibition of oxygen consumption of bird's red blood corpuscles produced by cyanides alone was not increased by the addition of narcotics (alcohols and urethanes) and that low concentrations of narcotics even decreased the inhibition The difference between my results and those of Warburg may be due to the fact that cyanides have a more powerful action in inhibiting oxidation than in inhibiting work, whereas the reverse is true for anaesthetics (Gray[16])

DISCUSSION

In the case of the action of alcohols and cyanides on the frog's heart there is no antagonism in the strict sense, since the addition of alcohol to cyanide or *vice versa* always produced a greater action than either of the components alone It is true, however, that in the presence of relatively high concentrations of alcohol, the additional action of cyanide was very small These facts can be accounted for by assuming that when the cells are exposed to a mixture of alcohol and cyanide, the alcohol occupies a certain proportion of the cell surface and thus limits the area upon which the cyanide can act This is in agreement with Warburg's theory that cyanide acts selectively on the iron of the cell But combinations of butyl chloral hydrate and alcohols produced the same effects as combinations of cyanides and alcohols, and therefore the displacement effect produced by alcohols is not peculiar to cyanides

SUMMARY

1 The relation between concentration and action of alcohols on the excised frog's heart shows a slight curvature although the deviation from linearity is not very marked

2 Combinations of alcohols produce an action which can be interpreted as a simple additive effect, provided that the curvature of the relation between concentration and action be taken into account

3 The relation between concentration and action of butyl chloral hydrate resembles the relation seen in the alcohols

4 The relation between action and concentration of cyanides is not linear, but appears to follow a hyperbola

5 Combinations of alcohols with either butyl chloral hydrate or with sodium cyanide do not show simple additive effects, but the effects observed can be explained on the assumption that the alcohol covers a portion of the surface and that the other drug acts on the remainder of the surface

I am much indebted to Prof A J Clark for suggesting this work, and for his help and advice throughout its execution.

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THE INFLUENCE OF TEMPERATURE ON THE MECHANICAL RESPONSES OF CERTAIN UNSTRIATED MUSCLES

By F R WINTON

(From the Department of Pharmacology, University College, London)

Introduction Among the few recorded observations on the variations of the responses of plain muscle to stimulation at different temperatures, those of Schultz[1], confirmed by Eckstein[2], are usually taken as characteristic They showed that the ring preparation of the frog's stomach yielded contractions increasing with temperature up to an optimum value Short faradic stimuli were employed Stewart[3] showed a similar effect of temperature on the contractions of the cat's bladder In a previous communication[4] the effect of temperature on the isometric response of the dog's retractor penis was shown to be closely similar, faradic stimuli of 5 seconds or shorter duration were employed

I know of no observations sensibly divergent from this relation of temperature and response in unstriated muscle, excepting an early account, by Grunhagen and Samkowsky[5], of the properties of various mammalian plain muscles, including particularly the rabbit's rectococcygeus They found that variation of temperature, between 20° C and 30° C , had little or no effect on the isotonic responses to short faradic stimuli

Experimental The following experiments were intended to define the region of optimum temperature for the isometric responses of the retractor penis to *prolonged* stimulation The muscles were stimulated in the same constricted tube apparatus, and with the same technique, as described in the record of earlier experiments[4] The tube was immersed in a bath of about 15 litres The temperature of the water in the bath was raised by running in warm water at the same time as running out the colder water, and lowered by an opposite arrangement Bubbling air was adopted as a stirrer, and appeared to be sufficiently effective, as accuracy of temperature exceeding ½ degree Centigrade was not required Faradic stimulation, at frequencies varying in different

experiments from 5 to 40 interruptions[1] per second, was employed
The stimulus was usually allowed to continue until the maximum
tension development had been passed   In certain experiments, the
duration of stimulus was constant throughout the series of contractions
at different temperatures   Fig 1 shows the record of a typical experiment
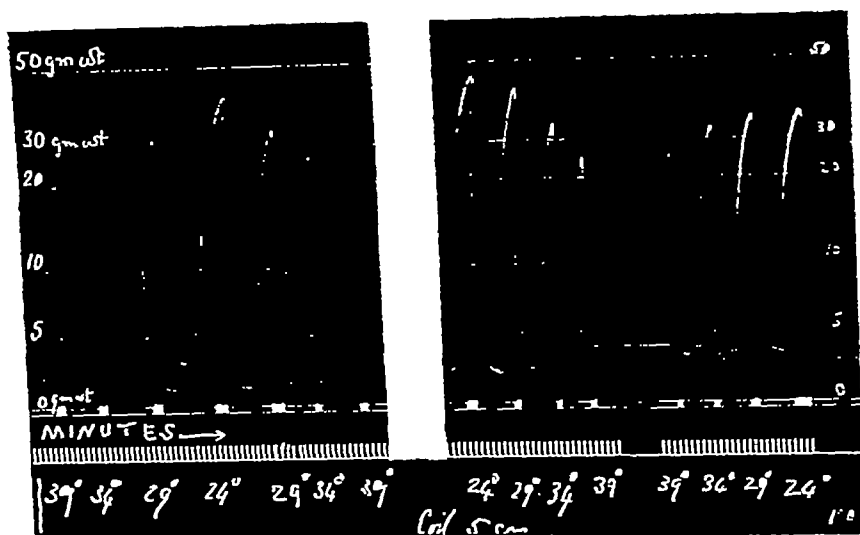
Fig 1   Isometric responses of a retractor penis to prolonged faradic stimulation at various
temperatures.

The results of this and similar experiments are given in Table I   They
show that both the total tension of the stimulated muscle, and the
tension developed on stimulation, decrease with increasing temperatures
between 19° C and 39° C   Below the former temperature, the un-
stimulated muscle tends to contract spontaneously, and to maintain
a relatively large tension for an indefinite period   Variation of contrac-
tion at a given temperature, according to whether that temperature was
approached by warming or cooling, was not constant in direction   All
these experiments were performed on muscles slowly stretched to nearly
their length of optimum response   An experiment on a muscle at
different temperatures, and at different lengths, suggested that the
optimum length was only slightly less at lower temperatures   The
preparation of frog's stomach, described by Schultz, does not appear

[1] Each interruption of the primary circuit induces a make and break shock in the
secondary   Consequently, the frequency of stimulation is double the frequency of inter-
ruption.

TABLE I Isometric response of retractor penis (dog) to prolonged faradic stimulation in Ringer solution. Palmer coil. 2 volts in primary circuit

| Muscle number | Stimulus | | Temperature °C | Tension in gm. wt. | |
	Intensity (position of secondary coil)	Frequency (interruptions per sec)		Resting muscle	Stimulated muscle
III.	4 cm	10	26 0	1 6	16·0
			31 0	1 0	12 7
			34 0	2 7	12 2
			38 5	3 4	5 7
			33 5	5 5	12 0
			28 5	5 5	16·0
			23 5	6·3	17 0
III	5 cm.	40	23 0	5 0	16·7
			28 0	3 2	8 7
			33 0	3 2	4 5
			28 0	4 0	13 1
			23 0	5 2	16·7
V	5 cm	5	39 0	2 1	20 5
			34 0	2 1	25 5
			29 0	1 0	32 0
			24 0	2 8	42 7
			29 0	1 1	34 5
			34 0	1 7	27 0
			39 0	2 6	21 5
V	5 cm	20	24 0	2 5	46 7
			29 0	1 8	43 2
			34 0	2 7	34 6
			39 0	3 8	25 4
			39 0	3 9	27 4
			34 0	3 7	32 8
			29 0	3 3	35 7
			24 0	3 2	37 0
II	7 cm	40	39 0	1 7	4 5
			39 0	1 7	3 8
			34 0	1 9	9 8
			34·0	1 5	5 6
			29 0	2 2	9 1
			29·0	2 9	10 0
			24 0	3 3	14 4
			24 0	3 5	11 6
			29 0	1 7	2 7
			34 0	1 3	1 5
			39 0	1 2	1 3

to show increased responses at lower temperatures, even with prolonged stimulation

The isometric and isotonic responses of the retractor penis, at different temperatures, to stimulation with adrenaline are recorded in Table II The contractions decrease with increase of temperature The isotonic response to 1 10^6 adrenaline changes only slightly in this sense,

TABLE II. The mechanical responses of the retractor penis (dog) to stimulation with adrenaline

Stimulus (Concentration)	Temperature °C.	Tension in gm. wt.	
		Resting muscle	Stimulated muscle
1 10^6	37 5	1 5	7 0
	37 5	1 5	7 5
	33 0	1 5	9 3
	28 5	1 5	21 5
	28 5	2 2	18 4
	23 0	6 0	30 0
	18 0	19·0	54·0
	23 0	3 9	44 0
	28 5	2 0	25·0
	33 0	1 0	18 5
	37 5	1 5	7 5
2 10^5	34 5	8 6	17 3
	30·0	8 6	20 7
	25·0	8 6	25 0
	19 0	10 0	25·7
	25 0	7 3	23 3
	30 0	7 3	15 5
	34 0	8 0	12 2

Stimulus (Concentration)	Load	Temperature °C	Actual length in cms	
			Resting muscle	Stimulated muscle
1 10^6	9 gm.	35 0	6·00	3 40
		28 5	5 82	3 34
		24 0	5·82	3 28
		18 0	5·40	3 20
		24 0	5·61	3 34
		28 0	5·82	3 48
		33·0	5·43	3 76
		38 0	4 90	4 24
1 10^7	8 gm.	34 5	5·6	4 0
		27 0	6·2	3 75
		41 0	6 7	5·0

and it is possible that a fully maximal dose would induce shortening to about the same length at all temperatures within the range under discussion The concentration of adrenaline which elicits a maximal isometric response is considerably greater than that which produces maximal shortening, it has not been employed in these experiments because it appeared to induce incompletely reversible consequences

Table III shows variation with temperature of the isometric responses of rat s duodenum to pilocarpine and adrenaline The tension of the muscle, stimulated with pilocarpine, tends slightly to decrease with increasing temperature The tension of the muscle inhibited by adrenaline appears to be substantially independent of temperature, though the

TABLE III　Isometric responses of rat's duodenum.

Stimulus Pilocarpine	Temperature °C	Tension in gm wt	
		Resting muscle	Stimulated muscle
1　40000	32 0	5 7	16 3
	27 0	7 0	16·1
	22 0	8 5	16 7
	27 0	10 0	15 2
	32 0	6 2	13 7
	37 0	5 5	12 4
Adrenaline			
1　10^6	37 0	8 5	4 8
	31 0	10 7	4 5
	26·0	11 5	4 6
	22 0	11 5	4 5
3　10^6	22 0	10 6	4 6
	27 0	10 2	4 7
	32 0	8 0	4 0
	37 0	6·9	4 0

negative tension development diminishes with increasing temperature, owing to progressive relaxation of the resting muscle

Conclusions　The retractor penis of the dog responds to prolonged faradic stimulation by developing less tension the higher the temperature This relation may be connected with the more rapid progress of fatigue in a series of responses at a higher than at a lower temperature, or with the increasing tendency of the unstimulated muscle to contract spontaneously at the lower temperatures　The increasing response to short faradic stimulation with increasing temperatures is probably due to shortening of the time relations of the contraction cycle, so that, the higher the temperature, the greater the proportion of the cycle during which a stimulus of constant duration is effective

The isometric and isotonic responses of the retractor penis to adrenaline decrease with increasing temperatures　That this simple relation between temperature and response is not uniformly found with other plain muscle, is shown by the isometric responses of the intestine to pilocarpine and adrenaline, and by the responses of the frog's stomach to electrical stimulation

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THE HYDROGEN ION CONCENTRATION OF THE MUSCLES OF THE CAT

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THE work described in this paper is the continuation of that already published by one of us (P M T K) with Katz and Long[1]   The hydrogen ion concentrations of both skeletal and cardiac muscles of the cat under nearly resting conditions, after electrical stimulation to fatigue, in rigor mortis, and on the addition of known amounts of acid and base have been determined, and also the normal *p*H and the buffer curve of uterine muscle

Experimental Method   The cat was anæsthetised with ether and chloralose   For estimation of the normal *p*H, the gastrocnemius of one limb was dissected, excised and immediately plunged into liquid air   The thorax was then opened, the heart was cut out, the pericardium removed and the cardiac muscle likewise plunged, still beating, into liquid air   The experiments on the uterus were performed later on different cats, the treatment being similar to that of the other muscles   Previous to estimation, the muscles were minced in a small tissue mincer in the cold store   The minced tissue was placed directly into a glass electrode, and its *p*H measured immediately by the method described elsewhere[2, 3]   Although the actual determination was not carried out in the cold store it was complete in less than three minutes after the tissue left the cold store, and the amount of lactic acid formed in the cold muscle in that time would be inappreciable

For the rigor mortis figures, the muscles were kept at room temperature for 24 hours before the *p*H estimation

When it was desired to stimulate the muscles after dissection they were cut off from the circulation by suitably clamping, and electrodes were applied to the muscles directly   The frequency of stimulation with break shocks was about 100 per minute   The position of the electrodes

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in the muscles was frequently moved to avoid apparent fatigue due to local blocks When the muscles failed entirely to respond further to electrical stimulus, they were excised, dropped into liquid air, and treated as described for normal muscles

For the determination of the buffer capacity the muscles were weighed after freezing, and before mincing The minced tissues in this case were mixed with about 40 c c of ice-cold NaCl (0 9 p c) This dilution was found by experiment to produce no alteration in pH The mixture was kept in a beaker surrounded by a freezing mixture, and the surface was covered with a thick layer of liquid paraffin Small amounts of normal solutions of lactic acid or potassium hydroxide were added at will by means of a 1 c c graduated pipette, the tip of which was dipped under the paraffin After these additions, the mixture was stirred and a small sample was removed under paraffin for pH estimation, by means of a glass tube furnished with a rubber teat The estimation was quickly made, and the sample returned to the main bulk of mixture It was ascertained that equilibrium with the solid phase was complete by repeating the titration on material already once used, and showing that the first and second results coincided

The values of hydrogen ion concentration recorded are at $0°$ C The pH of muscles in rigor was usually determined at room temperature In eight cases, the pH of these muscles was measured at room temperature and at $0°$ C In this way a pH-temperature correction was found, and the remainder of the pH figures of muscles in rigor expressed at $0°$ C The higher the temperature, the more acid was the muscle

In the determinations on the cold muscles, which were not frozen, but whose temperature was never allowed to get above $0°$ C , some assumption had to be made as to the temperature of the glass membrane, in order to calculate the pH from the E M F It was found inconvenient to work with the entire electrode at $0°$ C , owing to condensation on it of moisture from the air The phosphate buffer solution on one side of the membrane being at room temperature, and the muscles at $0°$ C , it was assumed that the temperature was midway between these two temperatures This assumption we considered justified on the following grounds Walbum[4] states that the alteration of pH of phosphate buffer solutions with temperature is negligible We showed that the change in pH of $M/20$ potassium hydrogen phthalate between $0°$ C and $38°$ C was less than 0 01 pH Further, the pH of $M/20$ potassium hydrogen phthalate solution was measured with the phthalate at $0°$ and the phosphate solution on the other side of the membrane at room

temperature, the above-explained assumption as to temperature of membrane being made This agreed with the other determination (3 97) within the limits of experimental error

TABLE I

pH of muscles at 0° C.

	Resting	Fatigued	In rigor
Cardiac	$7\ 07 \pm 03\ (9)$	$6\ 56 \pm 08\ (12)$	$6\ 39 \pm 11\ (16)$
Gastrocnemius	$7\ 04 \pm 03\ (15)$	$6 \cdot 26 \pm 07\ (14)$	$6\ 02 \pm 07\ (15)$
Uterus	$7\ 42 \pm 12\ (8)$	—	—

_p_H temp coefficient of muscle $-\ 004$ _p_H per 1° C.

The figures given represent each a mean value, with the probable error of the mean, based on experiments the number of which is given in brackets The probable error of a single _p_H determination on blood by the glass electrode method has been shown elsewhere[5] to be only $\pm\ 01$ The probable error of a determination on muscle will not be very different from this figure, and the probable errors of the means given in the above table represent almost entirely the individual variation of these constants in cats

Buffer curves The effect on the hydrogen ion concentration of the muscles of adding acid or base are shown in Figs 1, 2 and 3 Fig 1 embodies the results of experiments on the cardiac and gastrocnemius muscles of eight cats Fig 2 shows similar results on the uterine muscle of eight (different) cats Fig 3 shows the change in buffering power of the muscles with _p_H, and is calculated from Figs 1 and 2 by taking the tangents to the curves at intervals of 0 05 _p_H

A slight modification of the unit of buffering power suggested by V Slyke[6] has been used The unit of buffering power adopted is the differential ratio $\frac{dB}{dpH}$, expressing the relation between the increment (in millimols per 100 gm of tissue) of base B (or acid) added to the tissue, and the resultant increment in _p_H Thus a tissue has a buffer power of 1, when 100 gm will take up 1 millimol of base or acid per unit change in _p_H

Discussion The _p_H of resting skeletal and cardiac muscle is the same within the limits of individual variation The mean _p_H 7 05 is slightly more acid than venous blood The latter was determined directly by taking blood from the femoral vein of an anæsthetised cat, with a paraffined syringe The _p_H of the venous blood was 7 66 at 0° C , and 7 31 at 38° C

Fig 1

Buffer Curves of Muscles of Cat at 0° C

⟶ Acid added, mM. p c ⟵ Base added, mM. p c
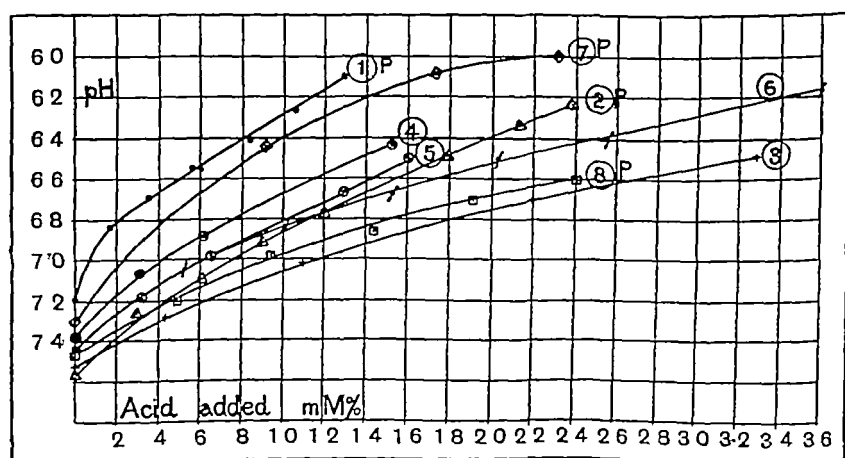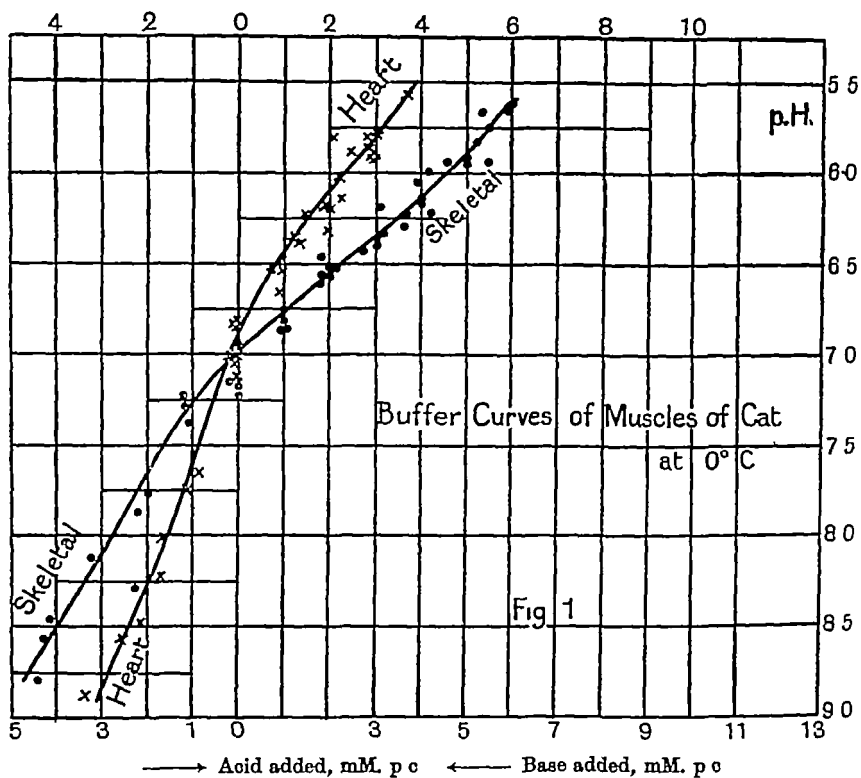
Fig 2 Buffer curves of uterine muscle of cat (P signifies pregnant)

For the determination at 0° C , the blood was cooled to 0° C under paraffin, and quickly transferred to the electrode The buffer solution on the other side of the membrane was at room temperature. The temperature of the membrane was assumed to be halfway between 0° C and room temperature For the determination at 38° C , the glass electrode and calomel electrodes were inside an electrically controlled air bath, so that the solutions on both sides of the membrane were at the same temperature, viz 38° C
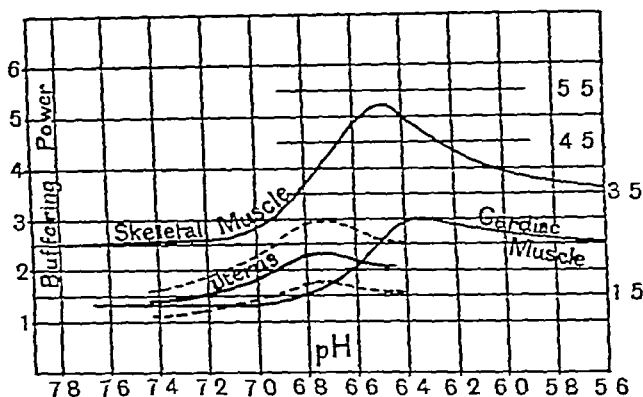
Fig 3

It is not easy to determine the temperature coefficient of the *p*H of resting muscles owing to the development of lactic acid above 0° C If it be assumed that the *p*H-temperature coefficient of resting muscles is approximately the same as that of rigid muscles, the *p*H of the freshly excised muscles may be supposed to be approximately 7 05–0 15, *i e* 6 90 at 38° C Katz and Long(7) found in their freshly excised muscles, which they plunged into ice-cold alcohol, 0 67 millimols p c lactic acid in skeletal muscle, and 0 33 millimols p c in cardiac muscle Part of this acid was unavoidably produced during the excision process It is probable that in our technique there was developed an amount of acid of the same order   From the buffer curve it can be seen that a subtraction of the above amounts of lactic acid from the respective muscles would give *p*H 7 30 and *p*H 7 28 at 0° C , or *p*H 7 15 and 7 13 at 38° C It therefore appears probable, if the assumption be correct that the *p*H-temperature coefficients of rigid and resting muscles are approximately equal, that slightly more acid was formed in our technique, than in that of Katz and Long This may have occurred either on

account of the liquid air freezing, or during the mincing Meyerhof and Lohmann(8) state that they found high resting values of lactic acid when they froze frog's muscles in liquid air

Combining the pH measurements of the muscles stimulated to fatigue and in rigor, and the buffer curves, it is possible to deduce indirectly the amount of lactic acid present in the muscles under these conditions, assuming that no acid other than lactic acid is produced during these processes The results of such calculations are given in Table II, together with the results found by direct experiment by Katz and Long For the purpose of comparison, pending direct experiments on the lactic acid formed during our technique, their resting values of lactic acid have been assumed

Considering the experiments were done on different animals, the agreement is very fair It may therefore be concluded that the above assumption was correct, i e that no acid other than lactic acid is pro duced in the muscles on stimulation, or after death

Meyerhof and Lohmann(8) found that the pH of frog's muscle at rest was 7 11, stimulated to fatigue was about 6 31, and about 6 0 pH in rigor These figures are very similar to those found by us for the skeletal muscle of the cat

TABLE II

	Lactic acid content (mM. p c.)	
	Direct estimation (K and L.)	Indirect estimation (F and K)
Skeletal muscle		
Resting	0 64± 13	(0 64)
Fatigued	3 0 ± 28	3 89± 20
In rigor	5 75± 52	4 94± 30
Cardiac muscle		
Resting	0 34± 02	(0 34)
Fatigued	0 85± 15	1 14± 20
In rigor	2 2 ± 30	1 64± 25

Meyerhof and Lohmann made an extract of the muscles with sodium chloride solution, water, or alcohol, and measured the pH of the extract with a hydrogen or quinhydrone electrode They also made a few experiments to determine the amount of change of pH on addition of acid, but their results are difficult to compare with our buffer curve on account of the fact that they used extracts instead of minced tissue They found considerable differences in the results obtained with different kinds of frogs, and with the same kinds under various conditions

The buffer curves of the heart and gastrocnemius muscles (Fig 1)

show points of inflexion. These inflexions are more obvious in the buffer curves of one individual than in a mean curve such as is reproduced here —the inflexions occurring at slightly different hydrogen ion concentrations consequently the effect is smoothed out in a curve through the experimental results on eight individuals

In Fig 3 where buffering power is plotted against *p*H, it will be seen that there is a maximum value for the buffering power of each tissue The titration curves are not accurate beyond *p*H 6 0 as in media more acid than this lactic acid is not fully dissociated. In an experiment on the buffering power of a beef infusion medium, Clark(9) found that the curves obtained, using lactic acid in one case and hydrochloric acid in another, were identical up to *p*H 6 0 In a few early experiments we used hydrochloric acid instead of lactic acid and obtained identical results

It can be shown that the hydrogen ion concentration, at which the buffering power is at a maximum, is numerically equal to the dissociation constants of the buffer acids(9) In muscles there will be a number of weak acids and their salts acting as buffers, and the K calculated thus will be a mean value This is equal to $3\,55 \times 10^{-7}$ for skeletal muscle, and $4\,47 \times 10^{-7}$ for cardiac muscle Thus skeletal muscle contains one or more acid radicles which are stronger than those of cardiac muscle and which raise the mean dissociation constant That there are several acids and their salts concerned in the buffering in this range of *p*H is also indicated by the fact that the buffering power $-p$H curve is not symmetrical, the buffering power being greater, at concentrations more acid than the maximum, than at concentrations to an equal extent more alkaline

The experiments of Andrews, Beattie and Milroy(10), on the buffering capacity of the extracted juice of skeletal muscle of horse and dog are difficult to compare with the results obtained by us as they do not give whole curves, but it is obvious that the buffering powers of the extracted juice of horse and dog and the minced tissues of cat are of the same order

The results with the plain muscle are noticeably different from those with the other two The resting *p*H is more alkaline, viz 7 42 assuming approximately the same *p*H-temperature coefficient as that of the other muscles in rigor this would correspond to 7 27 at 38° C , which is near the *p*H of the venous blood (7 31) The average deviation from the mean resting value was, however, about four times as great as in the case of the other muscles

The buffering power of the uterine muscle is remarkably different from that of the other muscles Especially noticeable is its inconstancy, whereas all the observations obtained on the gastrocnemius and cardiac muscles fall closely round a mean line, in the case of the uterine muscle all the points on any one cat lie on a smooth curve, but the curves on different cats show considerable divergence There is no obvious correlation between this variation and pregnancy (In cases where the uterus was pregnant the curves have been marked with a P) The curves in Fig 2 are somewhat difficult to compare, as the initial pH was different in each case In Fig 3 the mean buffering power of the uterine muscle is plotted against pH, and the average deviation from the mean curve on either side has been shown in dotted lines It will be seen that while the maximum average buffering power of the uterine muscle is not as great as the maximum buffering power of either the cardiac or the gastrocnemius muscle, yet in the range of pH from 7 4 to 6 6, the mean buffering power is greater than that of cardiac muscle, and in individual cases may be as great as that of the gastrocnemius The position of the maximum in the buffer curve is also different from that of the other muscles, occurring at pH 6 8, giving a mean dissociation constant of the buffer acids in uterine muscle as $1 6 \times 10^{-7}$

The buffering capacity of the muscles is due in part to the inorganic salts present, but also (and probably mainly) to the proteins Variations of the buffering capacity may be caused either by changes in the concentration of the constituents, or by changes in composition Saxl[11] showed that the protein content of the three types of muscle was different In striated muscle he found that $\frac{2}{3}$ of the total protein was "soluble" protein, constituting the muscle plasma, whereas in cardiac muscle only $\frac{1}{3}$ was soluble, and in smooth muscle only $\frac{1}{4}$ Two proteins could be identified from the muscle plasma in striated and cardiac muscle, which have been called myogen and myosin Myosin was shown to be absent from smooth muscle It remains to be shown by future work whether there is any correlation between these differences in protein content and the buffering capacities

SUMMARY

The hydrogen ion concentration of skeletal, cardiac and uterine muscles of the cat under nearly resting conditions have been determined, and of skeletal and cardiac muscles after electrical stimulation to fatigue, and in rigor mortis

Titration curves of minced skeletal, cardiac and uterine muscles

have been obtained The mean dissociation constants of the acids acting as buffers in the physiological range of _p_H are $10^{-6\ 45}$, $10^{-6\ 35}$ and $10^{-6\ 80}$ for skeletal, cardiac and uterine muscles respectively

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ON THE ACTION OF PILOCARPINE

By T B HEATON and M H MACKEITH

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THAT pilocarpine stimulates the endings of the parasympathetic system is generally agreed what other action, or actions, it may have is still a matter of uncertainty Edmunds[1] claims a direct action upon some varieties of plain muscle Dale and Laidlaw[2] state that it stimulates the endings of the preganglionic sympathetic fibres Cushny[3] appears to regard pilocarpine as promoting the output of adrenalin while Stewart and Rogoff[4] and also Elliott[5] find that this does not occur, at least to any appreciable extent Salant and Kleitman[6] suggest that pilocarpine may have the effect of sensitising the sympathetic system

Confusion exists even with regard to the effect of pilocarpine on the blood-pressure the account given by Dixon and Ransom[7] seems the most detailed in the literature These authors say "On the injection of a few mgm of pilocarpine into the circulation of a dog or a cat, the vessels contract, while the heart-beat becomes slower and weaker   the blood-pressure may either rise or fall, depending on the degree of weakening of the heart and of constriction of the vessels   a rise of pressure is usual in the dog, but is seldom observed in the cat " One tracing of a "pressor response" to pilocarpine in the dog is given in the article referred to, showing a rise of pressure amounting to a few mm of mercury above the pre-injection level

The experiments to be described were concerned chiefly with the effect of pilocarpine on the blood-pressure they were all carried out on cats of medium size (1500–2500 gm weight, except where otherwise stated), and all injections were made in 1 c c saline into the right external jugular vein towards the heart

Thus administered to the anæsthetised cat pilocarpine, as is well known, provokes a fall of blood-pressure associated with, and probably entirely due to, vagus slowing of the heart While there is, with moderate doses of about 1 mgm per kilo, speedy recovery from this fall, the blood-pressure does not rise appreciably above the pre-injection level (Fig 1)
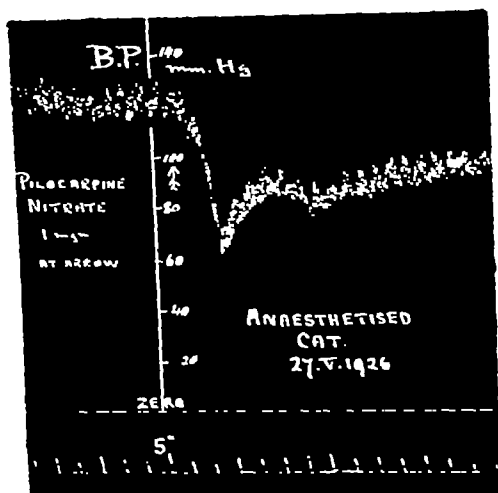
Fig 1.
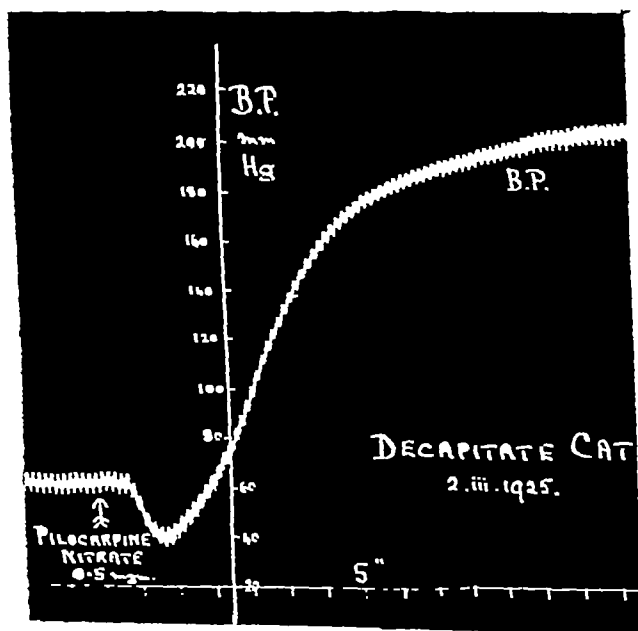
Fig 2

If, however, the injection be made into the decapitate preparation [8], the initial fall of blood-pressure is closely followed by a secondary rise, amounting often to as much as 100 mm Hg, and lasting for a considerable time (Fig 2) This consistent result has particularly attracted our attention, and to its attempted explanation the later experiments were directed

This secondary pressor effect cannot be attributed to any peculiarity of dosage, since it was obtained with doses varying from 0 13 to 6 0 mgm per kilo , nor to the cessation of anæsthesia, since it occurs unaltered when insufflation of the anæsthetic is continued through the respiration pump after decapitation, whether the "anæsthesia" be light or deep, and whether ether or chloroform Neither is the rise of blood-pressure an asphyxial effect, due to the broncho-constrictor action of pilocarpine as will be seen later, this is much less well marked in the decapitate than in the anæsthetised or decerebrate animal It will be seen more over from Fig 2 that the rise under consideration is not at all comparable in character with that arising from asphyxia

Neither is this pressor response to be associated with a low level of pre-injection blood-pressure It is true that as a rule the blood-pressure is lower in the decapitate than in the anæsthetised animal, but this is not invariably the case, and a rise after pilocarpine of 70 mm has been observed from a pre-injection level of 150 mm Hg (Fig 3), while in the anæsthetised animal we have never seen a pressor response, whether the initial blood-pressure were 130 mm or 60 mm.

Three different specimens of the alkaloid were used in the course of the work, and there was therefore no reason to suppose that the unexpected pressor effect was due to any deterioration or impurity in the drug

The pressor response is usually accompanied by some acceleration of the heart-beat above the rate existing during the preliminary vagal fall, but it is quite clear that the speeding-up of the heart is never nearly enough to account for the blood-pressure rise Indeed, quite often, there is some intermission of the heart at the height of the rise (Fig 5) It would therefore appear evident that the magnitude and persistence of the rise of pressure are dependent upon an action of pilocarpine on either the muscular walls of the arteries, or some point on the course of their nervous supply

If the suprarenal capsules of the decapitate cat be ligated before administering pilocarpine, the secondary rise in pressure is not obtained, or is but small in amount, the curve resembling that given by the
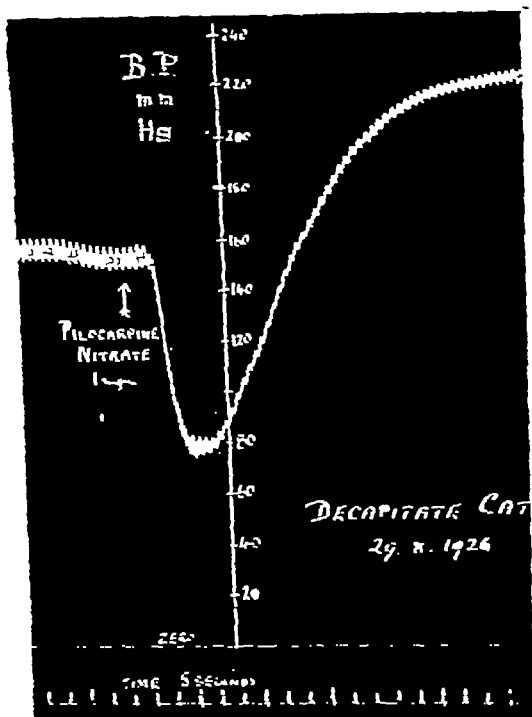
Fig. 3.
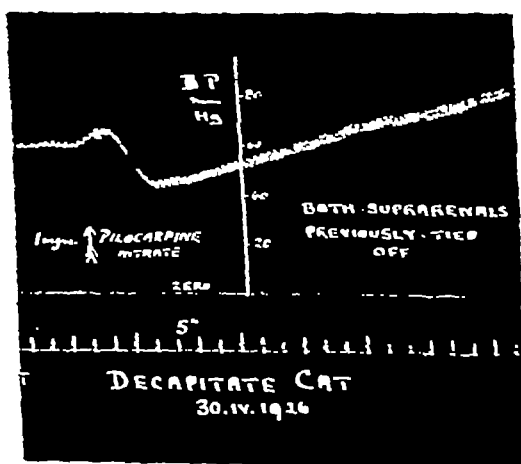
Fig 4

anæsthetised animal (Fig 4) This would appear to localise the action to the pre-ganglionic or ganglionic part of the sympathetic system, which of course includes the suprarenals it does not, however, necessarily indicate that the effect is due to liberation of adrenalin, since the operation of tying off the suprarenals involves considerable disturbances of the splanchnic nerves and abdominal sympathetic ganglia Indeed, Stewart and Rogoff(4) brought direct evidence that adrenalin is not liberated by pilocarpine injection (They were working, however, upon the intact and not the decapitate animal) Further evidence against the pressor rise being the result of adrenalin liberation is provided by (a) its long sustained character, and (b) the fact that it is cut short by atropine, in spite of the resulting cardiac acceleration (Fig 5) Cushny(3) also
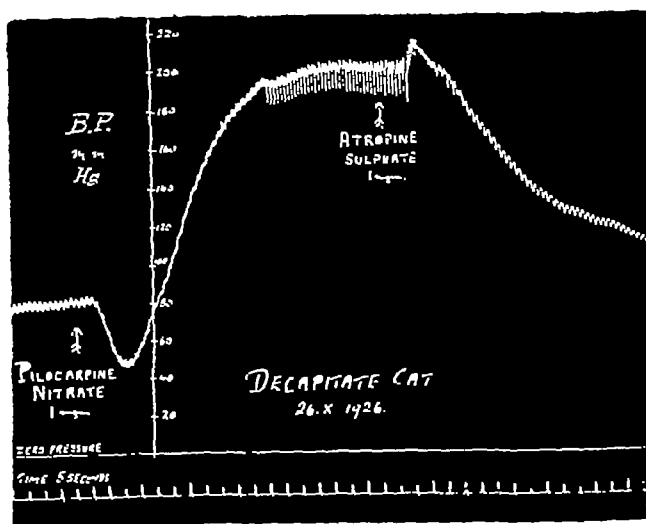
Fig 5

has noticed that the action of pilocarpine on the cat's uterus, which resembles sympathetic stimulation, is abolished by atropine

In order to test this point more accurately, pilocarpine was administered to a decapitate cat, whose sympathetic ganglia had been previously paralysed by apocodeine For this purpose nicotine is unsuitable, owing to its prolonged initial stimulant action nicotine further causes great reflex excitability, and depletion of the suprarenals Apocodeine has not these disadvantages in moderate doses, as Dixon(9) has shown, sympathetic ganglia are paralysed while peripheral vaso-

motor endings are unaffected and the suprarenals unimpaired. It was found that by injection into a cat weighing 2 kg of 100 mgm of apocodeine (10 mgm. at a time, at intervals of two minutes) a condition was reached where stimulation of the splanchnic nerve no longer caused a rise of blood-pressure The result of administering pilocarpine to such an animal is shown in Fig 6 the initial depressor action is slight, either
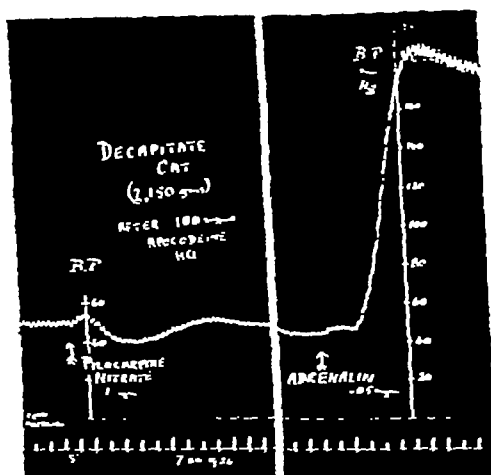
Fig 6

because the blood-pressure is already low, or because the vagus endings are paralysed (Dixon, *loc cit*), and it is followed by no secondary pressor effect at all The tracing shows also the effect of injection of adrenalin, proving that the peripheral effector organs are unaffected

From this experiment the conclusion was drawn that the secondary pressor action of pilocarpine on the decapitate cat can be attributed to action on the suprarenals only in so far as these form part of the system of sympathetic ganglia

When injections were made into the decerebrate preparation (10), breathing spontaneously and with medullary centres intact, pilocarpine was found to act as in the anæsthetised, and not as in the decapitate animal, there was no indication whatever of a secondary rise of blood-pressure (Fig 7) in fact the recovery occurring after the initial fall was usually less complete than in the anæsthetised animal Since it seemed possible that the secondary effect of the pilocarpine might have been masked by some potent depressor reflex, the experiment was repeated,

both in the anæsthetised and in the decerebrate animal, after section of both vagi The effect of the drug remained unaltered there was no pressor rise

It might be supposed, however, that there exists a *tonic* depressor influence of the bulbar vaso-motor centres, and that therefore these pressor responses can only be elicited after its exclusion This conception however would scarcely agree with (1) the fact that the blood-pressure in the spinal cat is definitely lower than in the intact animal, and not higher as would be the case had any depressor influence been removed in decapitation, and (2) the observation (cf Sherrington, *Mammalian Physiology*, pp 90, 143) that while

Fig 7

pressor responses can easily be evoked on stimulation of afferent nerves in the intact or the decerebrate animal, they are almost inelicitable on similar stimulation of the recently decapitate preparation

It would seem therefore (1) that pilocarpine has a pressor action on the decapitate cat, through direct stimulation of some point in the course of the sympathetic system, (2) that this action is only revealed when the connection of the bulbar centres with the peripheral mechanism (blood-vessel musculature) is interrupted, and (3) that since the integrity of the connection with the bulbar centres thus affects the result of administration, this action is exercised somewhere on the pre-ganglionic side of the vaso-constrictor path, and probably, as Dale and Laidlaw[2] suggested, at the endings of the pre-ganglionic sympathetic fibres

Repeated doses If a second dose of pilocarpine be administered to a decapitate cat immediately the effect of a moderate first dose has worn off, no pressor effect is obtained If, however, an hour be allowed to elapse between the two doses, a definite rise of pressure follows the second, though less than the very pronounced rise which follows the first Since Elliott[5] showed that such a phenomenon cannot result from suprarenal exhaustion, this affords further evidence that the secondary effect of pilocarpine is not due to adrenalin liberation It is not due to fatigue of the peripheral effector mechanism, for an injection of adrenalin immediately after the first pilocarpine dose produces its

normal result This absence of pressor effect with a second dose of pilocarpine would lend support to the view that the action of the drug is upon some readily fatigable part of the sympathetic nervous system.

Action on the bronchi Pilocarpine, as is well-known, produces on injection into the anæsthetised cat a marked constriction of the bronchi In the decapitate animal, however, a distinct constrictor effect is rarely observed, and marked constriction does not occur Since the bronchioles are constricted by vagus stimulation and inhibited by sympathetic stimulation (or adrenalin), this observation falls into line with the suggestion that in the decapitate condition a sympathetic stimulating action of pilocarpine occurs

Action of arecoline Though arecoline, weight for weight, is a more powerful drug than pilocarpine, it is generally stated that the actions of the two are similar On injection of arecoline, however, into the decapitate cat, only the initial depressor response was found to occur, with no secondary rise whatever This was confirmed for doses of arecoline varying from 0 0025 to 0 5 mgm per kg of body weight From this it would appear that while arecoline shares with pilocarpine a stimulant action upon parasympathetic endings, it has none of the latter's effect upon the sympathetic In accordance with this suggestion is the fact that while pilocarpine, as stated above, acts but feebly on the bronchi of the decapitate cat, arecoline on the other hand produces a marked constriction

Summary

1 The fall of blood-pressure which follows on the injection of pilocarpine is succeeded in the case of the decapitate cat by a secondary rise of remarkable extent and considerable duration

2 This secondary pressor effect is not seen in the decerebrate or in the anæsthetised animal

3 There is reason to believe that this pressor effect is due to an action upon the endings of the pre-ganglionis sympathetic fibres (Dale and Laidlaw), disclosed only when their central connections are divided This factor is held to account in some measure for discrepancies in previous results

4 Arecoline does not give rise to this secondary pressor effect, and the supposed identity in action between pilocarpine and arecoline is therefore not complete

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which he has granted us in his laboratory, and for much very helpful criticism

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REACTION OF SMOOTH MUSCLE TO THE H-ION CONCENTRATION

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THE reaction of smooth muscle to change in the H-ion concentration has engaged the attention of many observers. In 1923 Evans and Underhill[1] showed that the addition of dilute acid always caused a relaxation and alkali a contraction. The reaction to small changes of pH has been investigated by Gaskell[2] and Bayliss[3], who both obtained vaso-dilatation in the frog with weak lactic acid, Gaskell also noted that alkalies contracted the arteries. Young[4] and Botazzi[5] confirmed these results by experiments on the mammalian intestine, the latter also demonstrated that alkalies caused an increase of tone

Observations on the reactions with a greater range of pH were made by Dixon[6], who stated that weak lactic acid produced relaxation of the frog s stomach and while a certain increase in strength merely accelerated this effect, solutions of 1/500 caused a rapid increase of tonus, with subsequent slow relaxation. Wild and Platt[7] found that acidity caused vaso-constriction in the frog but that very weak acid sometimes caused a preliminary dilatation. Farndon[8] obtained similar acid effects on the mammalian uterus and added that alkali augmented the tonus. Fleisch[9] confirmed the fact that slight and strong acidity caused smooth muscle to relax and contract respectively. Atzler and Lehmann[10] have investigated the reaction of the blood vessels to changes in the H-ion concentration and have drawn a curve relating the rate of perfusion to the pH of the perfusing fluid. They suggested the effect was due to a physico-chemical reaction. Contraction of smooth muscle with acid has also been obtained by Hooker[11], Ishikawa[12], and Fraenkel and Morita[13]

In these experiments we have attempted to investigate the reaction of smooth muscle to changes in the H-ion concentration, first to ascertain if the muscle reacts to acids and alkalies in the same degree as to drugs, and secondly to find if possible the mechanism which is

responsible for alterations in tonus or length with small and large changes of pH

Method A glass muscle chamber and frontal writing point were used, the remainder of the apparatus being similar to that already described (14) Ringer-Tyrode solution was used, but phosphates were omitted, partly to obviate precipitation in alkaline solution, but mainly to have sodium bicarbonate as the sole buffer To control the pH of the solution carbon dioxide was supplied from a gasometer at a constant pressure, the rate of bubbling being regulated by a metal tap A curve was drawn relating the position of the tap with the pH of the solution as determined by the Dale-Evans method By altering the position of the tap a constant pH could be maintained for as long as desired, a change of 0 5 pH in the acid direction took not more than five minutes and in the reverse direction from ten to fifteen minutes The major part of the former change occurred within two minutes, in the latter the rate of change was almost uniform throughout For large alterations of pH sodium hydroxide and hydrochloric acid were used The temperature of the fluid was controlled at 37° C by a thermostat

Experimental results The present series of experiments shows the effect of changes in pH upon the strips of gastric musculature which are capable of altering their condition of tonus In a later paper the reaction of strips of smooth muscle which are capable of rhythmic movement will be described and compared to other types of muscle The stomachs of rats, rabbits and cats were used in the experiments The choice of strips was facilitated by reference to the paper of Brown and McSwiney (14) in which a table was given demonstrating that a tonus gradient exists, the muscle of the cardia and fundus giving the greatest reaction to pilocarpine, and that from the region of the pylorus showing practically no alteration in length The tonus gradient was found to be the same for pH change as for the addition of pilocarpine, the fundus showing the maximal alterations in length To investigate the reaction of the muscle to small changes of the H-ion concentration, the solution was altered within limits of about pH 7 to pH 8   The experiments were always commenced at pH 7 5

In experiments on the fundus and upper body a moderate increase of pH produced contraction of the muscle, a moderate decrease of pH caused relaxation These effects are reproduced in Figs 1 and 2, the strips of muscle being taken from the fundus of the rabbit's and cat's stomach respectively A frontal writing point was employed so that a good picture is seen of the onset and decline of the effects of acidity

(Fig 2) The course of relaxation is at first rapid but becomes gradually slower, which would suggest that the change of pH of the solution is
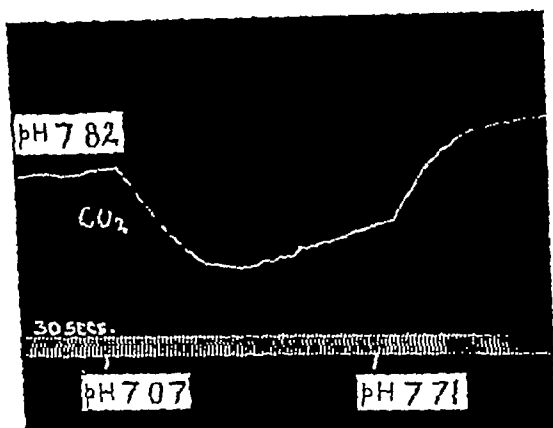
Fig 1 Rabbit's fundus. Tracing to show relaxation and contraction with moderate decrease and increase of pH.
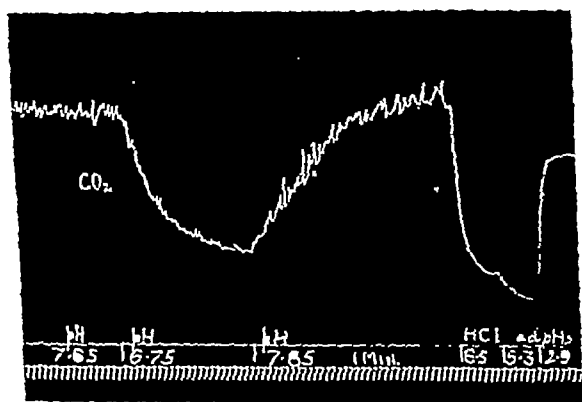
Fig 2. Cat's fundus Tracing to show relaxation and contraction with moderate change of pH, also contraction of the muscle with a big decrease of pH.

rapid enough to outstrip the rate of reaction of the muscle so that the latter stages of relaxation represent a gradual adjustment of the muscle to the pH The course of recovery, on altering the pH back to its normal value, seems at first to follow a straight line, this is probably due to the fact that the length of the muscle at any point corresponds to the pH

of the solution owing to the slow rate of increase in H-ion concentration
by the method of bubbling
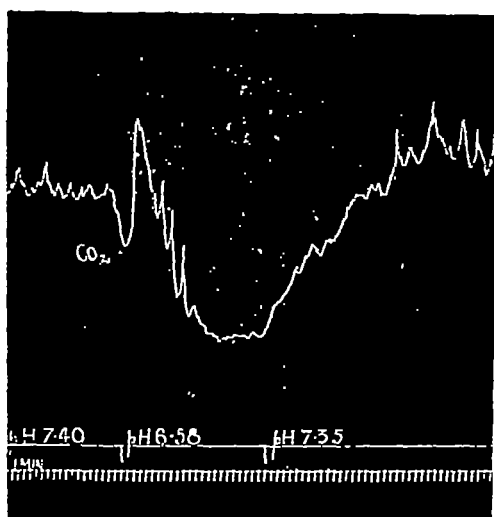
Fig 3   Fundus of cat. Tracing to show preliminary relaxation
and contraction on decrease of pH.

An interesting effect occurs in the fundic fibres of the greater curva-
ture of the cat and is, so far as has been ascertained, peculiar to that
region and animal.   On reduction of the pH a complete cycle of relaxa-
tion and contraction is gone through before the onset of the main
relaxation This phenomenon was so marked in one experiment that it
was mistaken for an effect normally observed with much greater altera-
tion of the H-ion concentration   Occasionally one or both phases in
the cycle may be replaced by a latent period during which the muscle
will show no alteration in length before the onset of the main relaxation

Another exception to the general rule was found in the cardia As
it was very difficult to obtain preparations of this part, owing to inclusion
of either fundic or œsophageal muscle, only a few experiments are
available from which any conclusions may be drawn These indicate,
however, that little or no effect is produced by moderate changes in pH,
whereas pilocarpine will cause a marked change in length When the
muscle is contracted after addition of pilocarpine the reaction to the
H-ion concentration becomes more marked

The contraction of the gastric musculature obtained with large doses

of pilocarpine was not final as the muscle could still be made to contract by a slight increase of alkalinity (Fig 4) The muscle can also be made to relax after the addition of atropine by slightly increasing the acidity
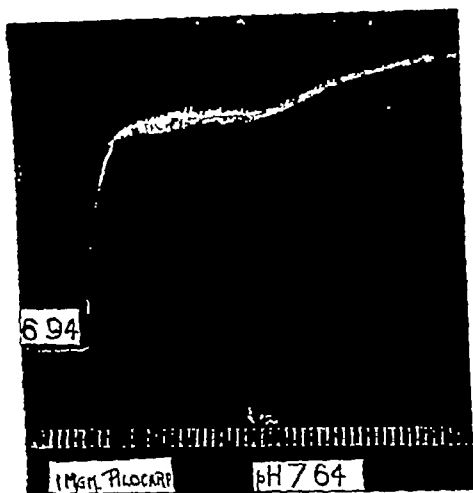
Fig 4 Preparation of rabbit's fundus Tracing to show further contraction of muscle with increase of *p*H after addition of pilocarpine.

After the study of the reaction of muscle strips to alterations of the *p*H within narrow limits, an attempt was made to see if these reactions were specific or merely one phase of a general acid and alkali action on the tissue For these investigations large changes in the *p*H of the solution were produced by addition of hydrochloric acid and sodium hydroxide, the *p*H being altered within limits of about *p*H 2 to *p*H 10 The experimental results are described in the order in which they were obtained The addition of 0 125 c c of hydrochloric acid to the solution caused relaxation of a strip of the rabbit's fundus, the final *p*H of the solution being 6 62 On altering the solution to *p*H 5 2 by further addition of the same amount of hydrochloric acid the muscle again relaxed, but increasing the acidity to *p*H 2 4 caused a sharp contraction followed by a slow relaxation

The same result is seen in Fig 5, where the first two doses were given in one When the acidity of the solution had been brought to *p*H 5 2 the muscle could be made to contract not only by addition of acid but also by addition of alkali The reaction of a strip of the rat's fundus, on addition of 1 c c of concentrated hydrochloric acid, sp gr 1 16, making the solution about *p*H 1 6, was a sharp contraction followed by

a slow relaxation, the muscle being prevented from showing the preliminary relaxation owing to the sudden large change in the pH of the solution   If only 0 5 c c of the same acid was added, making the solution about pH 2 18, then considerable preliminary relaxation was recorded followed by contraction   In later experiments it was found unnecessary to bring the tissue to the low pH of 2 4 to bring about contraction   The sudden drop of pH from 5 2 to 2 4 on the third addition of 0 125 c c of
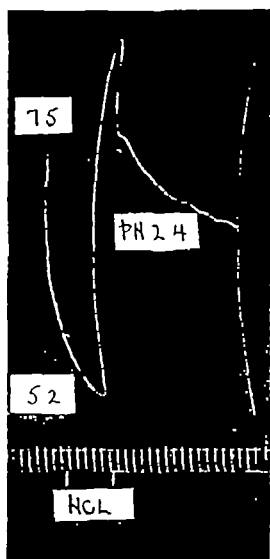
Fig 5   Tracing to show relaxation   Fig 6   Tracing to show contraction
and contraction on addition of HCl   of muscle at pH 5 9
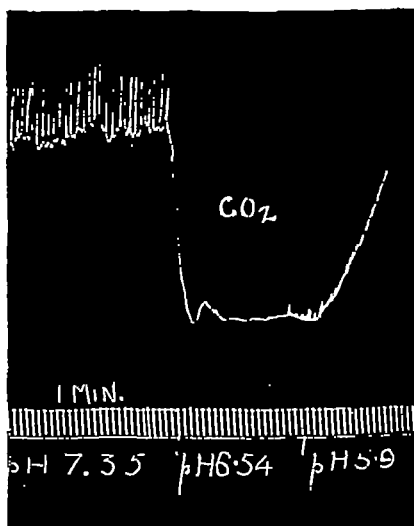
acid was due to the complete neutralisation of the bicarbonate, leaving free acid in the solution   Reduction of the pH to about 5 9, by the ordinary carbon dioxide method, is sufficient to send the muscle into a slow contraction   The rate of contraction varies as does the actual pH at which it occurs   Fig 6 was obtained from a late experiment, in which the technique was good, the muscle was free from mucous membrane and showed a rapid initial fall followed by a contraction considerably quicker than those obtained in the majority of experiments

A considerable number of experiments were performed with the object of finding the exact pH at which the relaxation would give way to contraction   The point, however, was found to be variable, and the most that can be said is that it nearly always occurred before pH 5 7

was reached, and usually at about pH 5 9   In a number of experiments contraction came on as early as pH 6 5, and in Fig 4 it can be seen commencing at pH 7   Sometimes too, even at pH 5 7, the muscle would remain relaxed without a sign of contraction   Although these are exceptional results, their presence, taken together with the varying rates of contraction of the strips, and their widely different latent periods, forbids more than a general statement as to the pH of the turning point The contraction when brought about by CO_2 is partially reversible, that is, a decrease in acidity will cause a certain amount of relaxation Further addition of acid after pH 2 1 has been reached causes a relaxation of the muscle

The effect of moderate changes of pH to the alkaline side have previously been described. the muscle contracting within normal limits On addition of NaOH to the solution the muscle contracted further, but after a certain point was reached a rapid relaxation occurred which
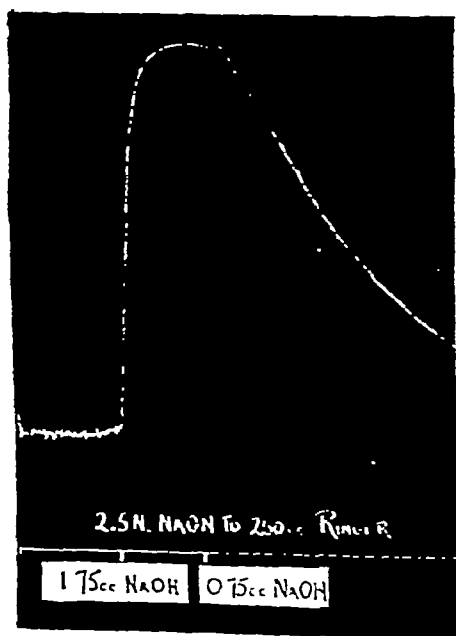
Fig 7   Rabbit s fundus   Tracing to show contraction and relaxation of muscle on addition of alkali.

was more rapid than the relaxation observed with acid after a final change of pH

In Fig 7 this result is seen with a preparation from the anterior surface of the rabbit's fundus when NaOH was added to the Ringer's solution so as to give the concentration 0 0175 N On further addition of alkali, to 0 025 N, a marked relaxation resulted It is possible there fore to obtain contraction and relaxation of smooth muscle on addition of alkalies, the former effect occurring within narrow limits

Discussion The curve shown in Fig 8 indicates the way in which smooth muscle, capable of a change in length, reacts when the pH of the solution is changed in a certain direction Whether the reaction is a contraction or a relaxation depends upon the pH at which the change is made It is necessary to bear in mind that all experiments were started at a pH of about 7 5, so that the curve must be read away from this
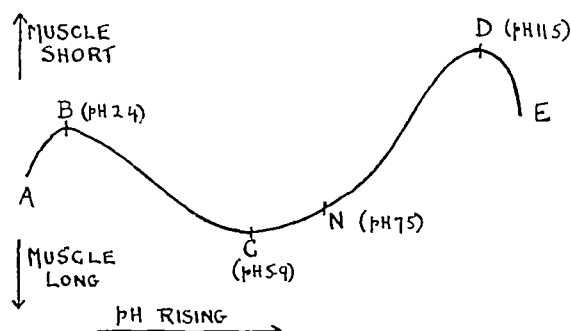
Fig 8 Curve to show reaction of smooth muscle with alteration of pH.

value, that is from the point N, on each side When the pH is increased the muscle contracts, as indicated by the line ND, but when the point D is reached further increase causes a rapid relaxation, shown by DE (Fig 7) On decreasing the pH a relaxation of the muscle occurs, corre sponding to NC on the curve, and this is followed by a contraction corresponding to CB (Fig 5) Further acidity brings about the slow relaxation BA (Fig 5) The turning points have been roughly determined, and B occurs at a pH of about 2, C at 5 9, and D at a strength of NaOH = 0 0175 N

In the region of CD changes of pH are almost completely reversible That is, after the tissue has reacted to a given change, it can be restored to its original length by reversing that change, and its sensibility is fully retained (Fig 2) Between C and B partial reversal is obtained with difficulty From B to A and D to E the change is irreversible

This curve, although not based on quantitative measurements, bears a

remarkable resemblance to Loeb s(15) curve showing the effect of pH on the swelling of gelatine This immediately recalls the suggestion of Atzler and Lehmann that H-ions act directly on the muscle protein. The fact that reactions to changes of pH can be superimposed upon the effects obtained with atropine, pilocarpine and nicotine suggests that the change in length is independent of the peripheral nervous system. This is further emphasised by experiments by Evans and Underhill and Atzler and Lehmann in which degeneration of the nerve plexuses was allowed to occur, allowing for the failing vitality of the muscle, the main reaction to pH changes was the same as in ordinary preparations Fleisch also demonstrated that vascular reactions obtained by central stimulation and those obtained by changes of the peripheral pH could be superimposed in mammals

Although our results suggest a purely physico-chemical basis for the reaction of the muscle to change of pH, it would be unjustifiable to attach too much significance to the mere shape of the curve It must be taken into account that only in the part CD is complete reversibility of action obtained, and if the system is a rigid physico-chemical one there would be no reason for one part of the curve to be different from any other in this respect Again, in this part, CD, the response to a change is prompt, and the end point of its effect definite, in other parts of the curve, the response is much more sluggish, and the effect produced is likely not to stop, unless interfered with, until it has gone absolutely to completion that is, when a further change of pH in the same direction would produce no continuance of the same effect Finally, there is the question of the variability of the point on the acid side where relaxation merges into contraction

These objections can be overcome by making the provisional assumption that the muscle protein is involved in some change which occurs in the other constituents of the muscle, when the pH is changed beyond certain limits On this basis the true equilibrium between muscle length and pH, observed in the part CD of the curve, would be an expression of the unfettered reaction of the protein to the pH changes The portion DE and BA would represent the involvement of the protein in some irreversible condition, in which it was changed so as to be insensitive to small variations of pH The part CB would then indicate the state of the tissue when the final irreversible change was creeping on, but the protein partly retained its original nature

CONCLUSIONS

1   Moderate changes of pH to the acid side of pH 7 5 cause a relaxation, to the alkaline side a contraction of smooth muscle

2   Large changes of pH to the acid side cause a contraction (pH 5 9 to 2 1), finally relaxation (beyond pH 2 1), of the smooth muscle   To the alkaline side, large changes (NaOH to 0 0175 N) cause contraction, and still larger changes finally relaxation

3   The reaction of the smooth muscle to alterations of pH appears to be independent of the peripheral nervous system

4   The reaction of smooth muscle to changes of pH, it is suggested, is of a physico-chemical nature

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THE SPINAL REFLEXES OF THE SKATE

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THE skate is an Elasmobranch, belonging to the genus *Raja* It is a relatively hardy animal and suitable for experimental studies The "spinal" skate is easily prepared and can be kept alive for weeks Its reflexes are numerous and lend themselves well to observation

Technique Observations were made on 25 skates caught by trawl and removed to the laboratory tanks which contained running sea water The skates were largely *Raja erinacea* Mitchill and averaged 20 inches in length They remained in the tanks two days, at the end of which time they appeared normally active Without removing the skate from the water, we cut between the medulla oblongata and the cord, entering by a small transverse wound in the mid-dorsal line immediately behind the most posterior part of the skull Hæmorrhage was controlled by plugging the wound with cotton

In 50 p c of the cases, the respiratory movements continued normally In the other 50 p c they ceased or appeared weak and irregular In the latter cases, we inserted a tube of running water into the mouth and allowed the stream to run out over the gills Normal respiratory movements having returned within an hour we removed the tube In a few cases we destroyed the brain, respiratory movements never then returned, the tube method of artificial respiration being used the whole time

Observations were made with electrical, thermal and chemical stimuli, but the mechanical proved so satisfactory and convenient that it was adopted entirely Both ventral and dorsal surfaces of the skate were investigated by pinching with metal forceps, and by light touch and stroking with the same instrument

These experiments are summarized in Table I, which should be read in conjunction with Figs 1 and 2

The response to stimulation of the posterior pectoral border (Area *E*, Fig 1) is bilateral, usually a little weaker on the contralateral side

TABLE I   Reflexes of the Spinal Skate in Response to Mechanical Stimuli.

Receptive area	Stimulus	Response
Ventral surface		
(1) Abdominal region (*A*)	Stroking or pinching the skin	A general ventral contraction of all the fins and of the tail
(2) At base of pelvic fin (*B*), area stretches out along the fin	Light touch	A ventral flexion of the distal part of the anterior lobe of the pelvic fin (unilateral)
(3) Pectoral fin—mid ventral surface (*C*)	Stroking	Curling of the edge of the fin ventrally
(4) Tail—ventral surface	Stroking and pinching	Strong posterior rotation (adduction) of the pelvic fins so that their posterior lobes overlap across the mid line in front of the tail
(5) Lateral border of pectoral fin—anterior half (*D*)	Pinching edge	Curling of edge of fin ventrally When stimulus is repeated the curling is followed by a vigorous dorsal flip of the whole fin
(6) Lateral border of pectoral fin—posterior half (*E*)	Pinching edge	Ventral curling of pectoral fin—anterior rotation (abduction) of anterior lobe of pelvic fin—a lateral movement of tail to side of stimulation
(7) Pelvic fin—tip of anterior lobe and posterior border of anterior lobe (*F*)	Pinching	Ventral flexion of distal part of anterior lobe When stimulus is strong—there is also a ventral curling of the pectoral fin
(8) Lateral border of posterior lobe of pelvic fin (*G*)	Pinching	Rotation of pelvic lobe—generally in a posterior direction but sometimes the posterior rotation is followed by anterior rotation which position is maintained
Dorsal surface		
(9) Mid line—particularly in region of pelvic girdle (*J*)	Stroking heavy	Dorsal arching of the tail and raising of the anterior part of the body, so that the pelvic region and the tip of tail only touch bottom of tank
(10) Soft part at the base of the pectoral fin (*K*)	Light touch	A dorsal curl of the pectoral fin
(11) Ditto (*K*)	Stroking	A ventral curling contraction
(12) Tail	Pinching whole thickness of tail	Lateral movement of body and tail to same side
(13) Dorsal fins and tip of tail	Pinching	Lateral movement of body and tail which, if the stimulation is continued alternates from side to side and is accompanied by rotatory movements of the pelvic fins The whole reflex if strong causes the skate to swim forwards

The lateral border of the pectoral fin curls strongly in the ventral direction, the anterior lobe of the pelvic fin rotates in the anterior direction (abducts) and often flexes ventrally The tail moves to the
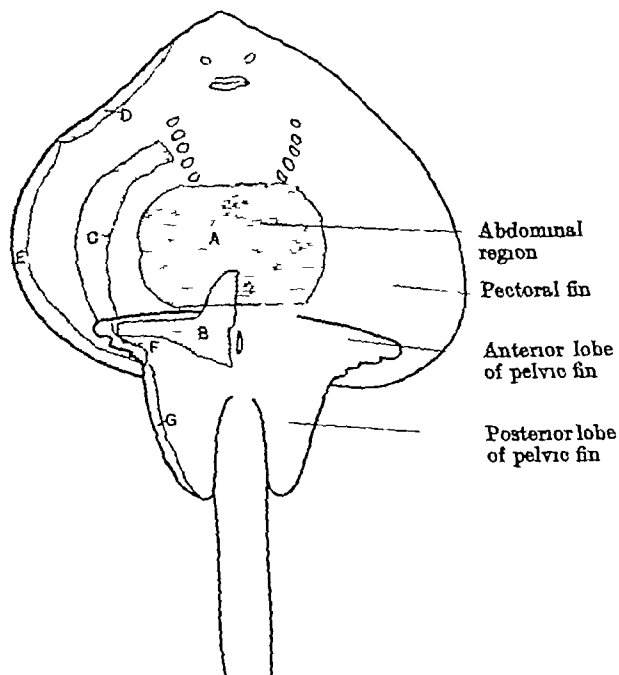
Fig 1 Areas of receptivity on ventral surface

ipsilateral side A pinch of the edge was the commonest stimulus used, but when the skate is very reactive a light tap suffices The reflex after-discharge is sometimes very long (1–5 minutes) It can be inhibited by the posterior rotatory reflex arising from stimulation of the tail It is among the first to appear and the last to disappear The response appears first in the pectoral fin, then in the pelvic fin, and later in the tail, and disappears in the same order When the response of the tail is very strong the contralateral pelvic lobe is often drawn down in a posterior direction When the skate is lying on its ventral surface the response of the contralateral fin is often an undulating wave of contraction, similar to that seen in the pectoral fins when the skate is swimming

The dorsal surface is much less sensitive than the ventral Comparison of the two responses from area K (Fig 2) is interesting The response to light touch can only be elicited after a number of stimuli

(8–12) are given   If stimulation is discontinued the pectoral fin arises only a few centimetres and then falls, but if it is continued the whole
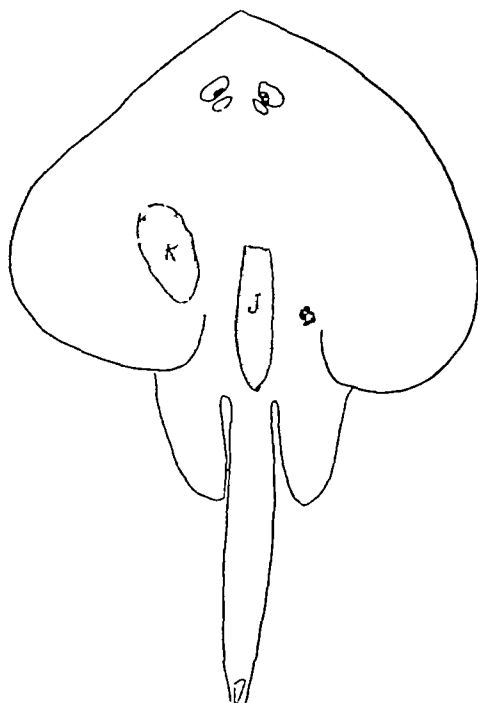
Fig 2   Dorsal surface

fin will make a complete curl so that the lateral edge touches the mid-dorsal region The response to stroking in the same area is by ventral curling which tucks the peripheral part of the fin under the rest of it In two skates the stroking had to be very severe or the response was similar to that from touch stimulation

I detected no period following the spinal operation in which all reflexes caudal to the section were absent The reflexes were usually weak, increasing in strength gradually for the first hour Extreme reactivity was observed 1 to 3 hours after operation, a very light touch then elicited a response This extreme sensitivity gradually disappeared and the skate then showed the regular responses as listed above

Immediately after operation, if the skate were held up by its tail, the pectoral fins fell down in a limp manner so that their borders almost met in the mid-ventral line The normal skate so held flaps these fins,

or if quiescent they hang in a more lateral posture with the peripheral part lifted dorsally In the second week after operation, the fins gradually recover this posture

With care the spinal skate would live for months I took no aseptic precautions in my operations The skate is extremely sensitive to anoxæmia One spinal skate lying in a small tank of still water exhibited very weak responses in three-quarters of an hour and total absence of them in two hours When the taps were turned on again the reflexes recovered and lasted some days

One skate showed active responses at the end of six weeks I fed the skates with a little fish muscle, putting the food into the back of the mouth Digestive operations appeared normal Normal egg-cases were laid by three of the spinal skates

Life habits I studied the swimming and feeding habits of the skate for possible relations of the reflexes to these activities of the normal animal

The skate, unlike the shark and other members of the *Raja* group, is a sluggish animal living on the bottom of the sea Its mouth is on the ventral side of its head It obtains its food (molluscs, crustaceans and smaller fish) by approaching them quietly, and swimming right over them It pins its prey down between its own body and the sea-bottom, and by quick movements of the fish fins, the prey is carried by water currents to the mouth and devoured I have observed that when one attempts to lift the skate up by the tail, it often resists with a strong hold of the fins on the bottom of the tank Sometimes in drawing up the trawl, a very severe tug is necessary to bring the skate from the bottom, showing that the flexor muscles must be very strong

There seem to be two types of locomotion In one the anterior lobes of the pelvic fin are used against the bottom of the tank, usually together but sometimes separately, while the pectorals exhibit undulating movements In the second and more rapid type of swimming the whole fish is off the bottom of the tank The anterior lobes of the pelvic fins are held far posteriorly, and the locomotion is carried out by the rhythmic undulations of the *pectorals* The tail is used as a rudder, but in rapid swimming it may be "swished ' from side to side In turning, the head end of the animal and the tail approach towards the side of turning—the pelvic fins being usually used against the bottom

Investigating one *Raja radiata*, I found strong reflexes which varied in certain ways from the responses of *Raja erinacea* For example, the pectoral edge reflex elicited a posterior rather than an anterior

response in the pelvic fins, which were shorter and thicker than in Erinaceæ

Anatomical notes   The pectoral fin border can be divided by its innervation into two parts which correspond roughly with receptive areas D and E (Fig 1)   The anterior part is supplied by the most anterior pectoral fin nerves, which, after passing out of the vertebral canal, form a "brachial" plexus   The posterior half is supplied by nerves which remain distinct throughout their course and innervate successive small areas of the fin   They run in a postero-lateral direction across the soft part of the back (the region between the girdles) lying just external to the peritoneum   Reaching the metapterygium of the pectoral fin they divide   A small branch runs around to supply the ventral musculature while the two terminal branches run still postero-laterally on either side of the metapterygium, one supplying the dorsal and the other the ventral muscle layers   The pelvic fin is supplied by twelve nerves, six going to the anterior and six to the posterior lobe   The first nerves form a plexus of a simple kind

Distal electrical stimulation of the cut end of a pectoral fin nerve caused a movement of the corresponding part of the fin covering about three to five fin rays   Distal stimulation of the first two pelvic nerves caused anterior rotation of the anterior lobe, of the fourth and sixth posterior rotation of the same, while the last six nerves ventral curling of the posterior lobe

By central electrical stimulation of the cut ends of the sixth to the eighth pectoral nerves I obtained anterior rotation of the anterior lobe of the pelvic fin

Results of transection, total and partial, of the cord   The cord was exposed by removing the appropriate laminæ from the dorsal aspect   Experiments showed that the extent of exposure of the cord should be as small as possible   As the hæmorrhage is often considerable, bleeding from the muscles of the wound was controlled by cautery   In one very active skate the spinal column was opened to the extent of 3 to 4 inches   The animal lived four days, but never recovered active reflexes   With exposure of the cord just sufficient for operation, the reflexes recover in 10 to 15 minutes

In choosing my level for transection I regularly counted eight vertebræ back from the pectoral girdle and entered posteriorly to the eighth, but there is much variation in the relation of cord to vertebræ

It is possible to isolate between complete transverse sections the part of the cord which contains the centres and receives the nerves of a par-

ticular reflex I have isolated the cord segments for the pelvic fin between two sections, one anterior to the first nerve to the pelvic fin, and the second twelve nerve segments further back Immediately after the final section, all reflex activity is lost, but begins to return in 10 minutes

A pinch of the edge evoked rotation and ventral flexion of the anterior lobe of both ipsilateral and contralateral fins In addition the back arched and the tail moved slightly This movement of the tail must be entirely due to the action of the back muscles above the second lesion because pinching the tail caused a lateral movement of the tail, but no response in the pelvic fins

I was able to isolate the posterior pectoral curl in response to pectoral pinch, but never got any satisfactory isolation of the anterior pelvic rotation reflex in response to pectoral pinch

I cut one half of the cord between the most posterior nerves supplying the pectoral fin and the most anterior supplying the pelvic, and used the reflex from the posterior pectoral edge (Area *E*, Fig 1) to test the results

In my first experiments I did not allow for a recovery period and judged that all responses of the pelvic fin from stimulation of the pectoral edge of the injured side were eliminated, while those of the uninjured side remained strong ipsilaterally and weak contralaterally

After a short period, however, all reflexes began to return On the intact side, the ipsilateral response was strong, the contralateral weak, while on the sectioned side the ipsilateral was weak and the contralateral fairly strong

In one skate with very strong reflexes I made a right semisection in the usual place Ten minutes after the operation both pectoral fins responded to pinching of the edge by a strong ventral curl The only response of the pelvic fins was the ipsilateral response of the left (uninjured) side In an hour the responses of the pelvic fins from stimulation of the right side had begun to reappear The tail responded to stimulation on the left side but showed no response on the right In four hours the right side showed a strong contralateral pelvic fin response and a response of the tail, which however was always *away from* the side stimulated

After 24 hours the skate was in good condition. The tail always moved to the left (uninjured) side and the right pelvic fin rotated posteriorly Thus stimulation of the right (injured) side yielded a strong contralateral anterior rotation, a weak ipsilateral posterior rotation and a very weak movement of the tail to the contralateral side In response to stimulation of the left (uninjured) side I received a strong ipsilateral

anterior rotation of the pelvic fin, a weaker contralateral posterior rotation, and a remarkably strong lateral movement of the tail to the ipsilateral side This position of tail strongly curled to the left, right pelvic fin posteriorly rotated and left pelvic fin anteriorly rotated, was often held for a very long time It is illustrated diagrammatically in Fig 3 It could sometimes be elicited by abdominal stimulation
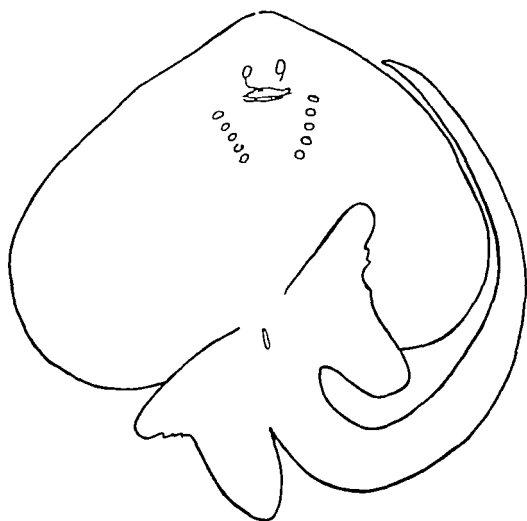
Fig 3

The pelvic fins were very sensitive Pinching their edge caused a posterior rotation of both pelvic fins, and a lateral movement of the tail to the stimulated side—thus the tail moved to the right side when the right pelvic fin was stimulated but to the left when the right pectoral fin was stimulated

By the fourth day the reflexes were weaker

The extent of spinal crossing of nerve-impulses is evidently considerable Microscopic examination of the lesion showed that the gray matter of the right side has been destroyed completely and the dorsal, lateral and a large part of the ventral columns of the white matter The ventro-mesial corner of the ventral column was not cut The left half of the cord was intact

SUMMARY

1   A "spinal" skate is a very suitable preparation for studying of reflexes

2   Different types of its spinal reflexes are described

3   Influence of total and partial transection of the spinal cord on the spinal reflexes is described

OBSERVATIONS ON THE MOTOR TWITCH AND ON REFLEX INHIBITION OF THE TENDON-JERK OF M SUPRASPINATUS

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THIS note describes some experiments carried out with the tendon-jerk of M supraspinatus in decerebrate and spinal cats, in comparison with the motor twitch, and offers some conclusions regarding reflex action deduced therefrom

Method of experiment The methods and apparatus used are precisely those which have been described in a recent communication (4) M supraspinatus has been isolatedly attached to a shadow myograph of high vibration frequency, and the muscle action currents have been sampled with the same string galvanometer (Cambridge pattern) The break shock has been applied from a Berne coil (coreless) in the manner previously described (1), the movement of the key which breaks the primary circuit (2 volts) being actually photographed on the plate For obtaining condenser discharges the "chronaximeter" of Lapicque[1] has been used The tap on the tendon has been given either by finger or by a cam-operated mechanical tapper devised by one of us (D D -B) The stimulating electrodes are of silver, coated electrolytically with chloride for condenser discharges, the kathode being remote from the cut end of the nerve and 1 cm from the anode When the motor nerve to M supraspinatus has been stimulated, the kathode has been 2 5 cm. from the muscle Nerves for stimulation are kept warm (37° C) and just moist with isotonic saline solution Spreading of the current (external and internal) along the nerve does not extend to the muscle as observed by the string record unless very strong stimuli are used (5 cm Berne coil) (15 A) The maximal and minimal twitches, described in the first section of the paper, need not have thresholds widely apart and are elicitable moreover with weak stimuli (23 cm maximal and 24 cm minimal, Berne coil, *i e* 1 cm above threshold) Neglecting the

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[1] Made by Messrs Boulitte, Paris.

brief period demanded for the passage of the impulse in this short nerve, their latent periods are $\pm 2\sigma$ for the maximal twitch and $\pm 4\,5\sigma$ for the minimal This difference may in part be due to current spread, but it appears largely to be a genuine and fundamental difference of functional activity, unless there be some very gross difference in the conduction rates of the nerve-impulse to the two types of muscle concerned Further evidence for this belief is forthcoming shortly(3)

The motor twitch In the investigation by the myographic method of reflex activities, it is necessary in order to draw any accurate conclusions from the isometric responses of the tendon-jerks to make a careful analysis of the isometric twitch resulting from the application of a break-shock or of the discharge from a condenser to the cut motor nerve This is manifest since the motor twitch is the absolute basic criterion of reflex activities

The maximal twitch of M supraspinatus has interesting properties There is a latent period of $= 2\sigma$ and a total duration, measured from the beginning of the action current to the "angle ' (Fulton(6)), of 36–48σ (Fig 1)—a very brief duration for mammalian muscle A well-marked "point of inflection" occurs relatively low down in the ascent which is evidence of the synchronicity of the volley in the motor impulse (5, 15)

The curve of relaxation from a maximal twitch of a homogeneous muscle declines regularly to the resting tension(7) The curve in M supraspinatus is seen, however, to show a more or less ill-defined secondary angle after half-relaxation, finally reaching the resting tension 200–280σ after the angle This secondary angle or "hump (13) we believe to be identical with the "Nase" of Funke(10) and the "contrazione lenta o tonica" of Bottazzi(1A) This delay or Nase is not due to low temperature or deficient blood supply(10), both of which we have been careful to exclude, but is the expression of a compound response of at least two categories of muscle fibre—red (slow) and pale (rapid) fibres An inspection of the anatomy of the muscle reveals a bulk of red fibres making up a considerable portion of its interior The more superficial fibres are distinctly paler

If a minimal twitch (Fig 2) be examined the curve of contraction, after a longer latent period ($\pm 4\,5\sigma$), is seen to have a low point of inflection, a less steep ascent, and a duration of 110–145σ, while the whole process still subsides within approximately the same time as does the maximal twitch There is no sign of the process which has the shorter latent period, ascent, and duration It is probable then that the

Fig 1   Fig 2   Fig 4
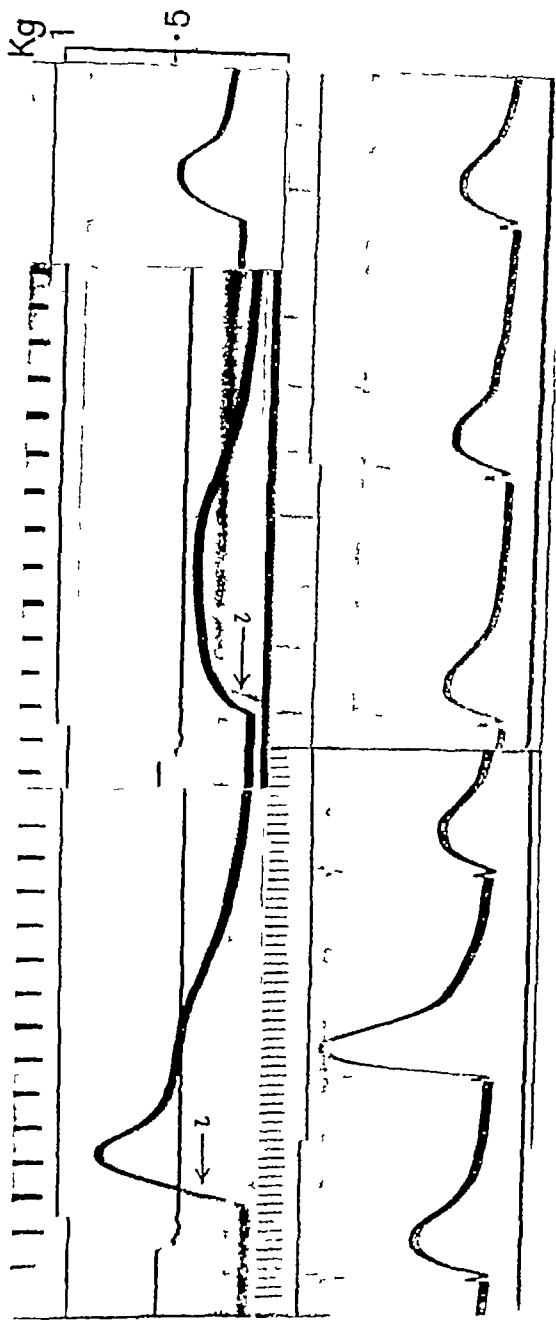
Fig 5

Fig 3

Time is shown at the top, 1 division = 20σ   Tendon movement multiplied 40 times   String tension adjusted to give 6–7 mm per 1 millivolt   ,   shows the point of inflection

Fig 1   A large motor twitch of M supraspinatus produced by a condenser discharge to the cut motor nerve   When the upper signal completes its upward crochet, the condenser discharges.

Fig 2   A small motor twitch, produced as in Fig 1

Fig 3   A series of tendon responses. The upper signal shows the application of a single break shock (Borne coil, coreless, 3 cm) to the ipsilateral ulnar nerve   Description in text.

Fig 4   A tap of less than 10 μ elicits a jerk developing 0 32 kg above the resting tension

Fig 5   A series of tendon responses   A break shock was applied to the ipsilateral median nerve at the crochet of the upper signal (Borne coil coreless 7 cm)

maximal twitch is the expression of the total combined response of the two types of fibres—which are known to differ in just those qualities which distinguish the two processes described above In the maximal response the angle of the "slow twitch is obscured because of the relative embedding of red fibres in the relaxing pale mass

There is some indication that fibres intermediate between red and pale may exist in supraspinatus—for instance a stimulus just supra-minimal occasionally shows a duration of 90σ

Introduction to observations on tendon jerk reflex The "knee-jerk" phenomenon is now generally accepted as a reflex action involving a very short intra-spinal path (Jolly[14, 15] and Snyder[20]) while the demonstration of its inhibition by appropriate stimuli (Sherrington[19]) had previously rendered indirect evidence that its character was probably reflex Jolly found that the latent period from the tap on the tendon to the current of action in the knee extensor muscle was only 5.5σ He postulated therefore the existence of only two synapses in the reflex arc

The general characters of the "jerk of M supraspinatus In our present experiments we have found that the latent period of the ' jerk ' of supraspinatus is of the same order of duration as for the knee extensor The intraspinal path must likewise be brief for this muscle also In Fig 3 it can be seen that the latent period from the onset of the tap to the registration of action current by the string is about 8σ Under varying conditions of reflex inhibitions we have found the latent period of the jerk to be lengthened or shortened according to the condition of the centre and we consider this as additional evidence of the reflex nature of the jerk Ballif, Fulton and Liddell[1] noted that a much smaller stimulus is needed to elicit the jerk-response in the decerebrate condition than in the spinal condition In these experiments on decerebrate M supraspinatus we also have found that very small stretch-stimuli (taps) can elicit a well-marked response Thus in Fig 4 the tap is approximately 0 3 mm as measured on the plate The combined optical and mechanical magnification of tendon movement in our present experiments is 40 times Therefore the stretch of the tendon is about 8μ Yet that is sufficient in this muscle whose longest fasciculi average some 60 or 70 mm and whose shortest some 26 mm to elicit a response developing 0 32 kg of tension

The decerebrate reflex response has a duration of from 80σ to 100σ (Fig 3) It is evident therefore that pale fibres must take part The reflex volley of impulses is less synchronous than is the motor, so that

the point of inflection is proportionately higher than in the motor twitch and less well marked (cf Fig 1 (motor) and Fig 6 (reflex)) The relaxation is slow and after inhibition occasionally shows a hump (Fig 5), this being most probably an enhanced myotatic appendage, since it is accompanied by a marked string deflection A less probable interpre tation is that the "hump" is a purely peripheral phenomenon ("Nase") resulting from the delayed relaxation of red fibres, in which case a string deflection would not be expected since it does not occur in the motor twitch

Behaviour to inhibition Single break-shocks applied to an ipsilateral afferent nerve, if suitably placed in time, can inhibit completely the jerk-response of supraspinatus in the decerebrate animal Similar con clusions regarding the knee-jerk were reached by Ballif, Fulton and Liddell(1) using precisely the same strengths of inhibition as in our present experiments A strong ipsilateral stimulus, however, has the additional effect on M supraspinatus of eliciting a large ipsilateral con traction This contraction, recorded both by the myograph and by the string movement[1], tends to complicate the observation of the inhibitory effect on the jerk The latent period of this ipsilateral single break-shock reflex is shown to be 66σ by analysis of records (Fig 6 A) The marked evidence of its presence at a period when the jerk is suffering extreme depression suggest that those muscle-fibres which engage in the jerk are different from those which are concerned in the single break shock reflex In Fig 6 B, for instance, the single break-shock reflex occurs on top of a jerk-response, while in Fig 6 c the reflex is reaching a plateau at the time when a tendon-tap recorded in the plateau elicits 40σ later a very small jerk-response It appears to be due to a number of flexor-like fibres ("flexor-like" in their response to an ipsilateral stimulus(9)) showing activity within a relative large mass of inert ex tensor muscle The angle of a small ipsilateral reflex is obscured by the damping effect of the remaining muscle mass Occasionally (Fig 6 A), a distinct "Nase" is seen, while the whole process has but a single initial electrical variation, suggesting that this is a response by both red and pale fibres to a single volley of impulses from the reflex centre

The general characters and time of appearance of the ipsilateral

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[1] We have satisfied ourselves that this single break shock reflex is a genuine pheno menon, and not an artefact due to general movement in the preparation, by frequent tests of our fixation methods and by direct observation of the tendon That primary movement of the string record is the same for the ipsilateral reflex as for the jerk supports the belief in its genuine character
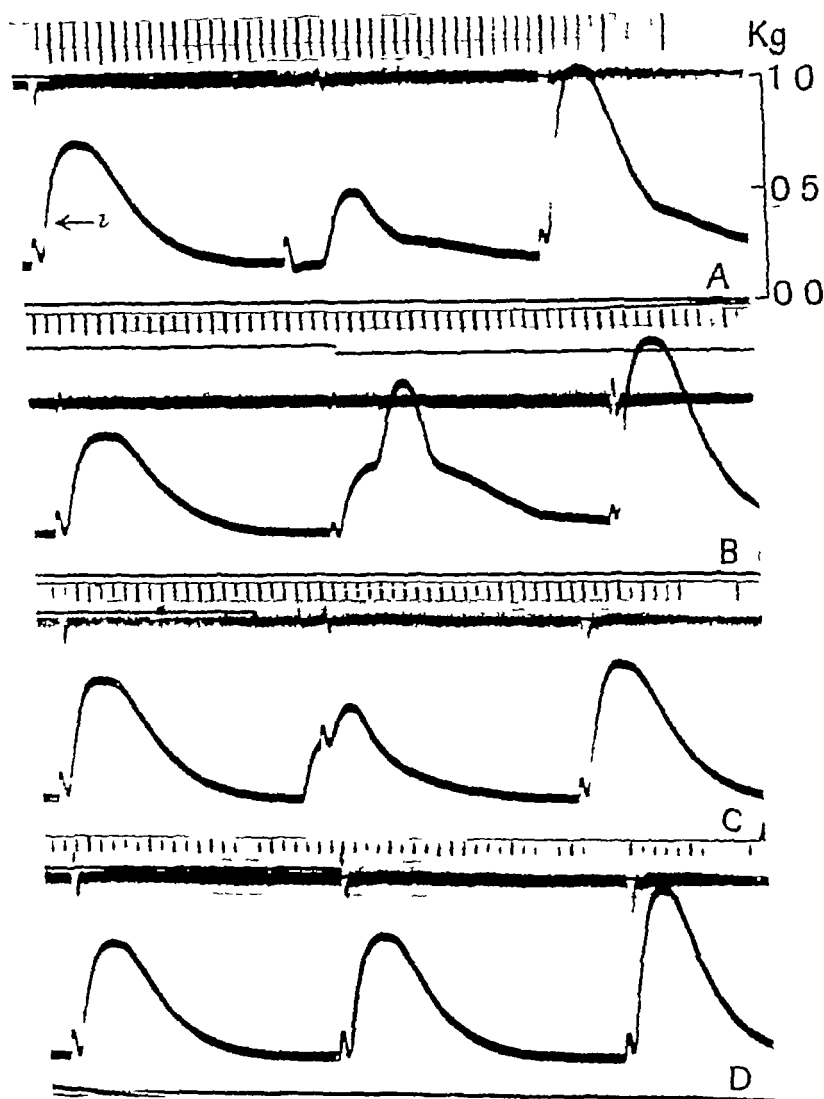
Fig 6 A A series of tendon responses. The signal shows the application of a break shock to the ipsilateral internal cutaneous nerve (Berne coil, coreless, 4 cm) B Same experiment coil 5 cm C D Same experiment coil 4 cm The signal is at the top of each record between the galvanometer string and the time marker

single break-shock reflex thus being established, it is possible to examine more closely the jerk-responses after inhibition by dissecting them away from the ipsilateral effect   As was adverted to above, Fig 6 A shows that when the inhibitory stimulus is almost coincident with the onset of the jerk, complete inhibition results   Fig 6 A therefore reduces to 1σ or slightly more the margin of time within which a single inhibitory stimulus, meeting an afferent volley *en face,* can annul it, a period with a brevity of the same order as that proposed by Ballif, Fulton and Liddell for the knee-jerk(1)   In Fig 6 D, the break-shock having been delivered to the nerve peripherally only 4σ before the efferent volley from the effector centres occurs, the inhibition arrives *too* late to cut short the central discharge which is already "under-way"

This phenomenon occurs also in the stretch-reflex of M supraspinatus A tetanic stimulus applied during the static phase of the stretch reflex may have only the smallest inhibitory effect   Yet the same stimulus, when applied before the stretch, is able to prevent entirely the develop ment of the reflex and the myograph tracing is that of a completely atonic or paralysed muscle   From this it may be concluded that in favourable circumstances inhibitory stimuli which can suppress the onset of a stretch-reflex may yet have only the slightest effect in the early stage of its actual course

Recovery from inhibition   This is a rapid process   In Fig 6 o the myo-graph and the string record give evidence that even within 100σ of the inhibition, there is some response to a tendon-tap   In another record there is more evident recovery within 110σ from a rather weaker inhi bition   Following immediately on the period of depression, there is a period of "rebound" (post-inhibitory exaltation, augmentation)   The time of onset and extent of this capricious phenomenon are variable Not infrequently in our experiments, as in observations by previous workers, its appearance is later after strong inhibitions, is earlier after moderate inhibition, and is absent after weak inhibitions   In the course of present experiments the "rebound" or augmentation at the central loci is entirely latent and does not become apparent until the state of the nerve-centres is sampled by a tendon-jerk   This latent exalted state at nerve-centres may, therefore, be a preliminary stage to the "rebound" contraction of previous authors   Thus in Fig 3 130σ after delivery of the inhibitory shock there is no alteration in the resting tension of the muscle   Yet a tap at that moment elicits a jerk of briefer latent period (5σ as against 8σ), of more rapid ascent and of shorter plateau accom-panied by a larger action current in the string   These data point to a

greater "wakefulness," as it were, of the nerve centres and to a more synchronous discharge of efferent volley of impulses This state of the nerve centres might be termed *latent successive induction* Figs 6 A, B and D show the same phenomenon with different strengths of stimulus and therefore with different times of onset (430σ in 6 A and D, 490σ in 6 B) In Fig 3 the exalted jerk shows an abbreviation in duration by some 20 p c as compared with the control jerks, and this not including the briefer latent period It is somewhat less abbreviated in Fig 6 A, B, D The stage of exaltation need not, however, be a true post-inhibitory phenomenon, but may be due to the stimulation of mixed nerves in the nerve-trunk. This has been adverted to in a recent paper[4] Whatever its fundamental character may be, it is an event which can take place up to some 500σ after a single moderate break-shock stimulus The knee-jerk and other tendon-jerks have often been accepted as evidence of the state of "tone ' existing in nerve-centres In Fig 3 the jerk is markedly enhanced without there being any increase in tone Nevertheless there is the exaltation of the tendon-reflex Sometimes there is actually a decrease of tone and yet exaltation of the tendon-reflex. It seems probable to us therefore that the tendon-jerk is not an index of the "tonus" of a muscle which latter is only an aspect of the so-called "stretch-reflex "

The exaltation following tetanic inhibition of the knee-jerk has been noted by Sherrington[19] and Viets[21] but was not observed by Ballif, Fulton and Liddell after a single break-shock inhibition It has been frequently recorded in our experiments on M supraspinatus That there should be this rebound or "swing-over" to exaltation after a single inhibitory stimulus is a phenomenon which recalls the appearance of negative after-images in the eye some time after withdrawal of the stimulus

In favour of the view that the apparent resistance of the nerve centres of M supraspinatus to inhibition may be due to the all-embracing electrical stimulation of mixed nerves, it has been observed that several brief stretches of the tendon of *M biceps brachii*, an antagonist, during 10 seconds may inhibit entirely the jerk-response of M supraspinatus for as long as 60 seconds This form of inhibition which is pure[2] and unmixed has been used to annul the stretch-reflex of M vasto-crureus in the lower limb[16]

This long lasting effect of the stimulus in its positive character is a contribution to the evidence that processes of inhibition or of excitation at nerve centres are states of considerable temporal duration,

not readily explicable by an interference mechanism in the nerve centres

The tendon jerk in the spinal condition In its general features, there is no very wide difference of the response, such as has been observed for the knee-jerk, in the spinal condition from that in the decerebrate A small tap is not usually so effective in eliciting a jerk in the spinal condition as it is in the decerebrate and it must often be twice as extensive This relative difficulty of elicitation was previously noted for the knee-jerk The latent period of the "spinal" jerk may be as brief as 6σ when the tendon-tap is sharp, or as long as 11σ In general, the latent period is 7–8σ and the duration of the response 85–95σ as measured from action-current to "angle" When the latent period is as long as 11σ, the response may have a duration of 120σ, suggesting activity of red fibres

There is then considerable variation in the latent period and duration of the jerks, which may obtain even in the same preparation This variation persists even when the tendon is tapped by the mechanical tapper, and every care taken to ensure constancy in the strength, direction and duration of the tap This variation has been noted by previous workers[17] In our experiments it is clearly not due to changes in tonic tension ("tone") of the muscle, as it is in the human subject[12] The mechanical tapper has enabled us, however, to deliver rapid second and third taps separated by only 3σ A second tap following so closely does not appear to add anything to the character of the response and therefore must fall within the refractory period of the jerk (cf 2A, 11)

Inhibition of the "spinal" jerk The "spinal" jerk can be inhibited by weaker stimuli than are needed for the decerebrate jerk Thus after a break-shock with the secondary coil at 19 cm complete inhibition of the jerk has been recorded In general, with stronger stimuli there is profound depression and a gradual recovery of the response such as Ballif, Fulton and Liddell recorded for the knee jerk ([1] their Fig 8) which is complete after one second or more But in M supraspinatus the variation already mentioned in a series of uninhibited jerks persists in the recovery phase after inhibition, thereby rendering difficult the plotting of points with any great degree of satisfaction Even with the strongest stimuli, however, there is usually some recovery of the jerk within 300σ, proceeding gradually to completion within 1500σ

In none of our records with the spinal preparation did we find any post-inhibitory exaltation ("latent successive induction") It was

interesting to observe also that in the spinal condition the ipsilateral contraction is lacking This confirms the findings of Bremer on that point (2)

SUMMARY

(1) Motor twitches of M supraspinatus recorded by the shadow myograph show activity of both slow and rapid muscle fibres The tracing therefore shows a distinct "Nase" (Funke) The rapid (? white) fibres may give a twitch lasting only 35σ The slow (? red) fibre twitch may last as long as 110σ

(2) In the decerebrate condition, the tendon organs may be exquisitely sensitive to a tap (stretch) of less than 10μ

(3) In the decerebrate condition, the tap can be inhibited by a single break-shock applied to the ipsilateral limb within 1–2σ A period of exaltation often ensues for as long as 1 second after the shock

(4) In the spinal condition, the inhibition is produced by weaker stimuli and is less quickly recovered from

(5) Both "quick" and "slow" muscle fibres may take part in the tendon-jerk, both in the decerebrate and in the spinal condition, with apparent preponderance of "quick" fibres

(6) The "ipsilateral single break-shock reflex" has a latent period of 60σ Both types of muscle fibre participate in it It is apparently a single volley reflex The reflex has not been found in the spinal preparation

(7) In the spinal condition, it has been found that a second tap delivered 3σ after the first does not alter or prolong the character of the tendon-jerk It is presumed that the second tap falls in a refractory phase

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NEGATIVE PRESSURE PULMONARY VENTILATION IN THE HEART LUNG PREPARATION

By I DE BURGH DALY

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MÖLLGAARD) has shown that the minute volume of the heart is increased when the thorax head, neck, and fore limbs of the dog are placed in a chamber in which the pressure is gradually reduced   In his experiments, the effective pulmonary arterial pressure was raised and there was an initial rise followed by a fall in systemic arterial tension as the chamber pressure was gradually reduced to -12 mm Hg   Möllgaard s technique, although being of great value in determining the effect of lowering the intrathoracic pressure upon the vascular system in the intact animal renders an analysis of the mechanism which brings about the vascular changes a difficult problem   A few experiments of a somewhat similar nature have been performed by the author, in which the heart and lungs were subjected to a progressively reduced pressure and the blood-flow taken through one lung lobe   Dogs were used under complete anæsthesia and the thorax closed after all operative procedure had been completed Ventilation of the lungs was carried out by rhythmically varying the intrathoracic pressure, the mean pressure being kept negative It was desired to investigate the effect upon the heart output of lowering the intrathoracic pressure in the absence of nervous influences This was not possible even when the heart was completely denervated and the phrenic nerves cut because variations in the pulmonary ventilation alter the composition of the blood and produce effects upon the medulla   For this reason, further experiments were carried out with Starling s(2) heart lung preparation, the heart and lungs being subjected to a reduced pressure which was of an oscillatory nature in order to produce expansion and collapse of the lungs The oxygen and carbon dioxide content of the blood in the heart-lung preparation remains tolerably constant over short periods, provided the pulmonary ventilation is adequate In addition, nervous influences are absent and the initial output of the heart may be varied at will

Method In all experiments dogs are used, fully anæsthetised with

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NEGATIVE PRESSURE PULMONARY VENTILATION IN THE HEART LUNG PREPARATION

By I. de BURGH DALY

(From the Physiology Institute, Cardiff)

MÖLLGAARD[1] has shown that the minute volume of the heart is increased when the thorax, head, neck, and fore limbs of the dog are placed in a chamber in which the pressure is gradually reduced   In his experiments, the effective pulmonary arterial pressure was raised, and there was an initial rise followed by a fall in systemic arterial tension as the chamber pressure was gradually reduced to − 12 mm Hg   Möllgaard's technique, although being of great value in determining the effect of lowering the intrathoracic pressure upon the vascular system in the intact animal, renders an analysis of the mechanism which brings about the vascular changes a difficult problem   A few experiments of a somewhat similar nature have been performed by the author, in which the heart and lungs were subjected to a progressively reduced pressure and the blood-flow taken through one lung lobe   Dogs were used under complete anæsthesia and the thorax closed after all operative procedure had been completed   Ventilation of the lungs was carried out by rhythmically varying the intrathoracic pressure, the mean pressure being kept negative   It was desired to investigate the effect upon the heart output of lowering the intrathoracic pressure in the absence of nervous influences   This was not possible even when the heart was completely denervated and the phrenic nerves cut, because variations in the pulmonary ventilation alter the composition of the blood and produce effects upon the medulla   For this reason, further experiments were carried out with Starling's[2] heart-lung preparation, the heart and lungs being subjected to a reduced pressure which was of an oscillatory nature in order to produce expansion and collapse of the lungs   The oxygen and carbon dioxide content of the blood in the heart-lung preparation remains tolerably constant over short periods, provided the pulmonary ventilation is adequate   In addition, nervous influences are absent and the initial output of the heart may be varied at will

Method   In all experiments dogs are used, fully anæsthetised with

chloralose, 0 1 grm per kilo body-weight being injected intravenously The pericardium is kept intact throughout the experiments, except for small openings which are necessary for the insertion of the pulmonary and auricular cannulæ The closed circuit heart-lung preparation is made in the manner previously described (3, 4), with the addition that the tubing connecting the heart and lungs with the external apparatus passes through a large rubber bung The preparation having been set going, the heart and lungs are removed from the body and placed in a large glass vessel, the rubber bung serving as a stopper The lungs are then ventilated by producing rhythmic variations in the pressure in the glass chamber, the mean pressure value being negative, *i e* below atmospheric pressure

In practice, the closed circuit heart-lung preparation is made with the rubber bung lying on the neck of the animal. The bung is pierced by a number of glass tubes, to which the rubber tubings to and from the heart and lungs and the external circuit are connected. The arrangement is illustrated in Fig 1, the course of the blood being through the channels
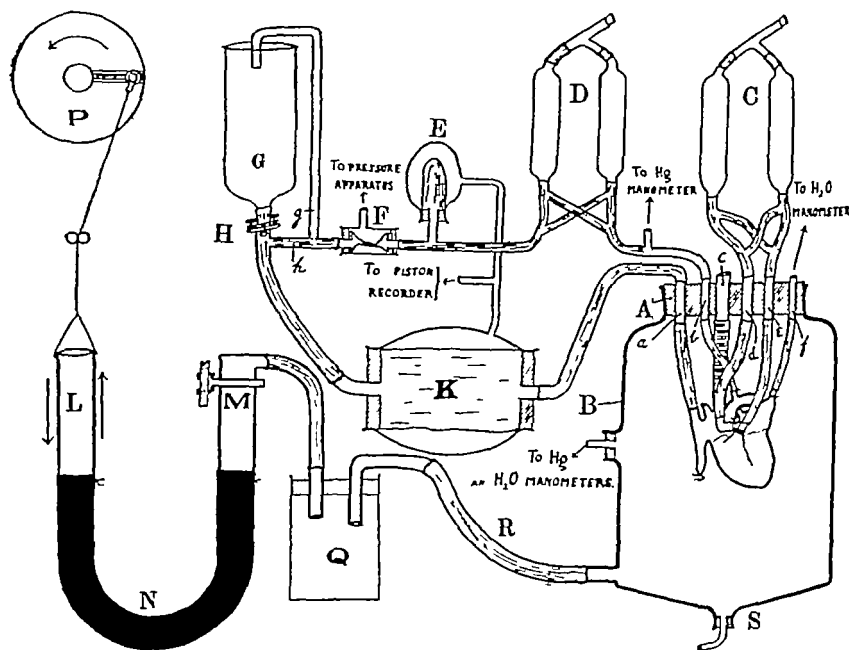
Fig 1   The heart and lungs are placed in the respiratory chamber *B* The lungs are not shown in the figure   For explanation, see text

marked *b D, E, F, H* and *K*. The tube *g* is clipped, and the reservoir *G* not in circuit The carcase is removed from the heart and lungs, a much easier proposition than removing the heart and lungs from the carcase, since the former are attached by short lengths of

tubing to the external apparatus which is a fixture In order to facilitate this procedure, the top of the operation table is made so that it can be removed by sliding it from underneath the heart and lungs which have been previously freed from their connections with the body The trachea is tied to the lower end of a wide bore tube (c) passing through the bung and then artificial respiration continued through this tube The preparation is suspended in a vertical position by gripping the bung in a condenser clamp The remaining portion of the trachea is of such a length that it carries the whole weight of the heart and lungs

For the purpose of recording the blood flow and pressures, cannulæ are inserted into the central and peripheral ends of the cut pulmonary artery These are joined by short lengths of rubber tubing to the lower ends of the glass tubes marked d and e, their upper ends being connected to a Pavlov and Stolnicov stromular (C, Fig 1) For recording the pressures in the pulmonary artery, right auricle and left auricle, cannulæ are inserted into a branch of the pulmonary artery, and into the right and the left appendices Connections from these pass through the bung to water manometers. The upper ends of the water manometers are joined to piston recorders The flow recorders having been filled with liquid paraffin, the heart and lungs are placed in the glass chamber and the rubber bung pressed tightly into the neck to make an air tight joint. All the connections within the chamber B are of stout walled rubber so that a reduction in the chamber pressure has no appreciable effect on their volume The glass vessel dimensions are as follows maximum diameter 18 cm. diameter at neck 10 cm. maximum height 30 cm.

Artificial respiration having been discontinued, the interior of the glass vessel B is placed in communication with the negative pressure apparatus marked P, L, M, Q, R (Fig 1) This consists of two brass cylinders 7 5 cm. in diameter and 28 cm. in length, one of which (M) is fixed and the other (L) movable in a vertical direction. The lower ends of the two cylinders are connected together by a large rubber tube (N) 5 5 cm. in diameter and 70 cm. in length, a portion of a Dunlop inner tube Water is poured into the open end of L until it reaches half way up the cylinders L and M, N being completely filled. An adjustable crank P imparts a vertical motion to L so that the water level in M rises and falls as the crank rotates. The top of the cylinder M is connected through a water trap Q to the glass chamber B by means of lead tubing with gas mask tubing junctions By adjustment of the crank stroke the rhythmic pressure variations in the vessel B can be altered at will, the mean pressure being controlled by varying the amount of air in the closed space B R, Q M A mercury manometer records the pressure changes in B, and an outlet tube (S) fitted at the lower end allows of the withdrawal of any blood which leaks from the preparation. The rate of respiration is regulated with the aid of an adjustable resistance in series with the motor driving the crank.

In some experiments it was desired to record the changes in blood volume of the heart and lungs. For this purpose the venous reservoir K and the elastic cushion in the systemic circuit are surrounded by plethysmographs connected together and placed in communication with a large piston or water volume recorder In this way the change in the volume of the external circuit can be observed, since the portions inside the plethysmographs are the only distensible parts of the external system Volume changes in the artificial resistance and in the rubber connections are found to be so small under working conditions, as to be negligible With this arrangement a given change in the volume of the external circuit denotes an equal and opposite change in the blood volume of the heart and lungs

Before commencing observations, the interior of the respiratory chamber is brought to atmospheric pressure The artificial resistance

and the volume of blood in the circulation are adjusted to give a peripheral blood-flow of from 300–400 c c per minute at an arterial pressure of approximately 90 mm Hg The chamber is then closed and air withdrawn through a side tube so that the pressure variations due to the pump are below atmospheric pressure A further reduction in the mean respiratory pressure is brought about by withdrawing more air from the chamber, the crank of the pump being unaltered, the difference between the in spiratory and expiratory pressures remains the same The four glass water-manometers are set up side by side, each fitted with a millimetre scale, so that the maximum and minimum pressures in the respiratory chamber, the pulmonary artery and the two auricles can be observed in about 45 seconds The observed values were, in the majority of experiments, also recorded with pistons attached to the upper ends of the manometers

Experimental results When the lungs are ventilated by rhythmic variations in the respiratory chamber pressure, variations in the venous and arterial tensions take place at each respiratory cycle (Fig 2) An examination of the ordinates in this and other tracings of a similar nature, shows that the fall in the respiratory pressure, producing inflation of the lungs, is not as a rule coincident with the drop in the arterial and venous tensions The factors which determine the time relations of the several pressures will be discussed at a later date, and only those effects due to a reduction in the mean respiratory pressure will be considered in this paper

Fig 2   Dog 6 5 kilos   Pericardium closed. *B P* =systemic arterial pres sure   *R.A* =pressure in right auricle *R P* =pressure in respiratory chamber The mean R.P is lowered from −5 to −12 mm Hg   *I* =inspiration. *E* =ex piration.

Naked eye observation of the lungs during their expansion and collapse do not give definite evidence of a change in their colour during the respiratory cycle, when the respiratory frequency is 15 per minute or more If, however, the pump is stopped with the lungs in the inspiratory position, the surface of the lungs take on a pinker tinge in the majority of experiments

Owing to the preparation being suspended in a vertical position, the upper lobes of the lungs tend to fall over to one side so that the blood supply and the air entry to these lobes is somewhat diminished, as evidenced by their paler colour and relatively smaller volume Sometimes this undesirable effect can be overcome by careful manipulation, but placing the whole preparation in a horizontal position would be, perhaps, a better plan

Effect of lowering the mean pressure in the respiratory chamber It has been found that certain precautions have to be taken to ensure that the changes which occur on reducing the respiratory pressure are really due to the pressure reduction and not to concurrent events The temperature of the heart and lungs is apt to fall considerably below that of the blood in the external circuit apparatus, because these organs are more exposed than when lying in the chest If care is not taken to equalise the temperature throughout the system, alterations in the blood-flow produce variations in the heart rate which complicate the results

A certain amount of blood leaks away and this gradually reduces the cardiac output(3), so that one is working on a falling base line The same phenomenon is also produced by the gradual pooling of blood in the lungs which has also been found by d e J a g e r(5) working on perfused lungs The blood leakage generally occurs at the rate of 3–4 c c per minute, although higher values than this are encountered In two experiments no external leakage occurred To obviate any gross errors in the interpretation of the results, the respiratory pressure is reduced in steps and then brought back to its initial value, observations being made at each step The results are plotted on squared paper and the level of the base-line determined

The usual effect of lowering the respiratory pressure is illustrated in Fig 3 Starting with the pressure at zero it is reduced in three stages to -150 mm H_2O, then brought to -120 mm. H_2O and finally to zero It will be seen that the lower the respiratory pressure the greater the peripheral output of the heart The pressures in the pulmonary artery and the left auricle, as measured by the water manometers, fall, but the effective pressures (the difference between the manometric and the respiratory chamber pressures) rise It is the effective venous pressures which determine the output of the heart, and in this connection P a t t e r s o n and S t a r l i n g(6) have shown in the heart-lung preparation that the same peripheral output can be maintained with a lower venous pressure (manometric) if the pericardial pressure is reduced L e w i s(7), using the whole animal, obtained an increase in diastolic filling of the heart by the

same means   All the effects shown in Fig 3 might be ascribed to an
increased diastolic filling alone   It is clear, however, from a series of
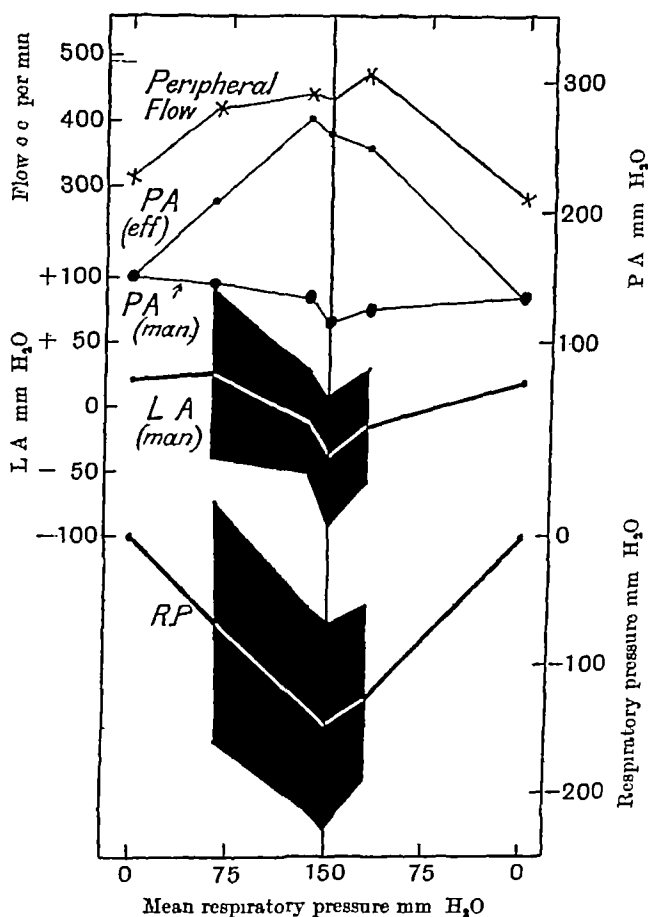
Fig 3   Dog 6 0 kilos. Pericardium closed   Heart rate 114 per min throughout. *L A*
=left auricular pressure   (*man*)=manometric pressure   (*eff*)=effective pressure
The maximum, minimum and mean auricular pressures are shown, also the inspiratory
and expiratory R P   Effect of lowering, then raising the mean respiratory pressure
Respiration rate = 14 per min

experiments which is not quite complete that the lungs play an important
part in the response of the preparation to a reduced respiratory pressure
     In the experiments of Patterson and Starling and of Lewis, the
pericardium was opened, the question arises, therefore, as to whether
a marked increase in diastolic filling of the heart can be obtained with

intact pericardium by a reduction in the respiratory pressure, steps being taken to eliminate changes in the pulmonary circulation. To test this point the following experiment is performed. An isolated heart preparation is made, the lungs being replaced by a Hooker and Drinker(8,9) blood oxygenation apparatus. This entails the tying off of the lungs after the heart-lung preparation is made and then the insertion of cannulæ into the central end of the pulmonary artery and into the left auricle. The apertures of the pericardium necessitated by this operation are closed by drawing the edges tightly round the cannulæ. The blood from the right ventricle passes through the rubber bung to the oxygenation apparatus and back again to the left ventricle. The isolated heart is then placed in the respiratory pressure chamber. The results of one experiment are shown in Table I, in which the increase in

TABLE I. Effect of a negative pressure on the isolated heart with pericardium intact

Pressure on the heart mm. H_2O	Output from right ventricle c.c per minute	M.B P mm Hg
0	320	96
− 25	325	97
− 60	340	99
−110	350	100
−170	365	102

A further reduction in the pressure on the heart reduced the output

cardiac output brought about by lowering the chamber pressure, is small but definite. The cause of the small change in output is probably due to (1) the pericardium being somewhat diminished in size owing to the incised edges being drawn together, and (2) the hypodynamic condition of the heart. Under the second heading, an interesting problem arises. In this and other experiments the isolated heart rapidly became hypodynamic in spite of good oxygenation of the blood. On mentioning this fact to Prof E B Verney(10), he informed me that he had carried out similar types of experiments and had arrived at the same conclusion. It would appear that the functional capacity of the heart is impaired when the lungs are removed from the heart-lung preparation which is running on defibrinated blood. The main point in question—whether the heart with pericardium intact increases its output when subjected to a negative pressure, may be answered in the affirmative.

Coronary blood-flow Running hand in hand with the augmented systemic output, there is an increase in the pulmonary flow (Fig 4). In this figure the pulmonary arterial tension base-line has dropped considerably, and the shaded area is drawn to accentuate the changes in

the manometric pressure The pulmonary and systemic outputs are
measured simultaneously As the blood level in the flow recorders passes
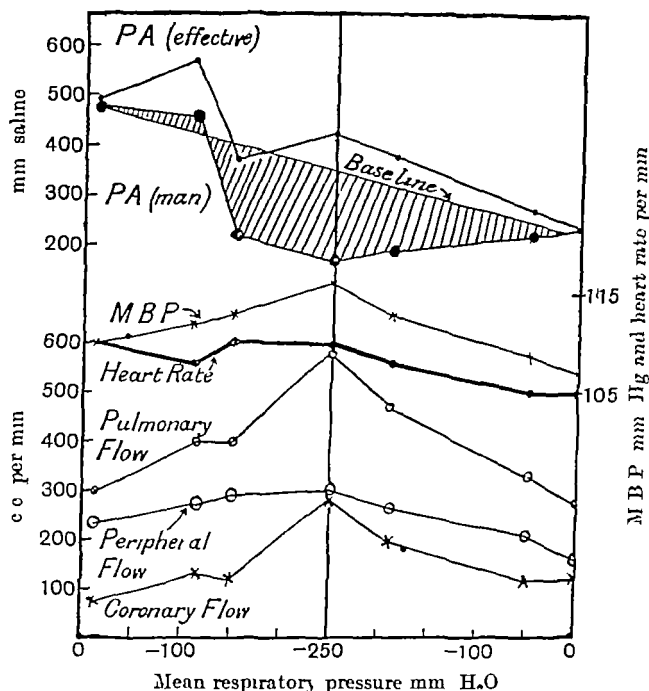
Fig 4 Dog 8 0 kilos Pericardium closed. Effect of lowering then raising the mean
respiratory pressure Respiration rate = 15 per min

the graduated marks, two contacts are made in two separate electrical
circuits, and these actuate two signal markers writing on the smoked
paper of the recording drum The coronary blood-flow is calculated as
the difference between the pulmonary and systemic outputs The differ
ence between the inspiratory and expiratory pressures has been varied
from 30 to 200 mm H_2O, 100 mm H_2O generally being used Within
these limits a reduction in the mean respiratory pressure increases the
output The intrathoracic pressures in the anæsthetised dog are given
by van der Brugh(11) in two experiments as −106 and −81, difference
= 25, and as −128 and − 68, difference = 60, all figures representing
mm H_2O pressure Wiggers(12) gives − 70 and − 34, difference = 36,
as average values from a number of experiments The difference between
the inspiratory and expiratory pressures in our own experiments have
in general been larger than those found in the anæsthetised animal,

partly because the lungs require a greater negative pressure in the heart-lung preparation than in the intact animal if the same amount of ventilation is to be obtained, and partly in order to gain some idea of variations in output which would be likely to occur as a result of changes in the intrathoracic pressure during exercise That the values used cannot be considered beyond physiological limits is indicated by the observations of van der Brugh, who, in confirmation of Ewald, found that with the tracheal air entry impeded, the inspiratory and expiratory intrathoracic pressures were − 1100 mm H_2O and + 1070 mm H_2O respectively, readings obtained during marked respiratory distress

The augmentation of heart output is found to take place in some experiments on reducing the respiratory pressure to − 400 mm H_2O, lower pressures than this have not been tried In other experiments, the heart fails before this pressure is reached because it is unable to deal with the increased inflow, if, however, the pericardium is opened and the heart allowed to dilate, then the output goes on increasing with the respiratory pressure lower than that obtaining at the time of failure Accepting those experiments in which the functional capacity of the heart is greatest, it may be stated that no evidence has been forthcoming that a negative pressure of 400 mm H_2O is, in itself, injurious to the heart when the pericardium is intact In this connection, Talma[13] considered that a markedly low intrathoracic pressure impeded systole and favoured diastole, however this may be, it is obvious that if the output is increased, systole will not be so complete and the diastolic volume will be greater, and these changes in themselves are not of necessity an indication of a diminished functional capacity of the denervated heart

Effect of heart rate and work of heart in relation to negative pressure ventilation With an initial output of 300–400 c c per minute and an arterial pressure of approximately 90 mm Hg, reductions in the respiratory pressure lower the venous pressures as recorded by the manometers If the work of the heart is greatly increased, the respiratory pressure being zero, then lowering the latter causes no change in the venous pressures or may even produce a rise In these cases the extra work imposed upon the heart by the augmented inflow raises the venous pressure (manometric) to the same extent as, or more than, the respiratory pressure reduces it A heart in poor condition generally shows a rise in venous pressure at comparatively low respiratory pressures, because the raised inflow produces a relatively large rise in venous pressure and because the more the heart is dilated the less effective does the respiratory

pressure become, since the pericardium is intact   Extracts from the
protocols of an experiment in which the venous pressure first falls and
then rises are given in Table II   In this experiment the peripheral output

TABLE II. Dog   Closed circuit heart-lung preparation. Negative pressure ventilation.
Heart rate=126 per min   Respiration=14 per min   Mean pressures in mm H₂O

L.A. (man.)	L.A (eff)	P A (man)	P A. (eff)	R P	M.P.B mm Hg
+ 10	10	105	105	0	73
− 4	69	36	101	− 65	85
0	95	14	109	− 95	94
+ 90	240	80	230	− 150	122
+120	365	113	358	− 245	132

measurements are absent, but the rise in arterial tension with constant
peripheral resistance and heart rate, show that the cardiac output is
increased

If a gradual reduction in the mean respiratory pressure is causing
a fall in the mean venous pressure, and then the peripheral resistance is
raised, the venous pressure shows a diminished rate of fall or even a
rise   The same effect accompanies cardiac acceleration (Fig 5)   Yas
Kuno(14), working with the heart-lung preparation with intact peri-
cardium, demonstrated that the maximal output of the heart is much
greater at the higher beat frequencies, and this has been repeatedly con-
firmed   It follows from Yas Kuno's work, that cardiac acceleration in-
creases the amount of work performed by the heart when the pericardium
is intact   In Fig 5, during the first period (A) the respiratory pressure
is being reduced with the heart frequency kept constant, the venous
pressures are falling   During the second period (B) the respiratory pres
sure is still further reduced, but the heart rate is accelerated (by raising
the blood temperature) and the venous pressures are rising owing to the
extra work thrown upon the heart over and above that caused by the
reduction in the mean respiratory pressure   In the third period (C) the
reverse changes to those taking place during the second period are
shown

DISCUSSION AND CONCLUSIONS

The assumption that the heart and lungs, suspended in a vertical
position, react in the same manner to a reduced mean respiratory pressure
as do the heart and lungs in the whole animal, is not altogether valid
Even if a thoracic respiration is defined as one due to movements of the
thorax with the diaphragm fixed, there are certain objections to this
assumption   In the whole animal the visceral and parietal pleuræ are
in apposition which is not the case in the experiments described   In this

connection, Keith (15, 16) has indicated the lines of force which expand the lungs, these lines do not lie radially from the lung roots as they do in
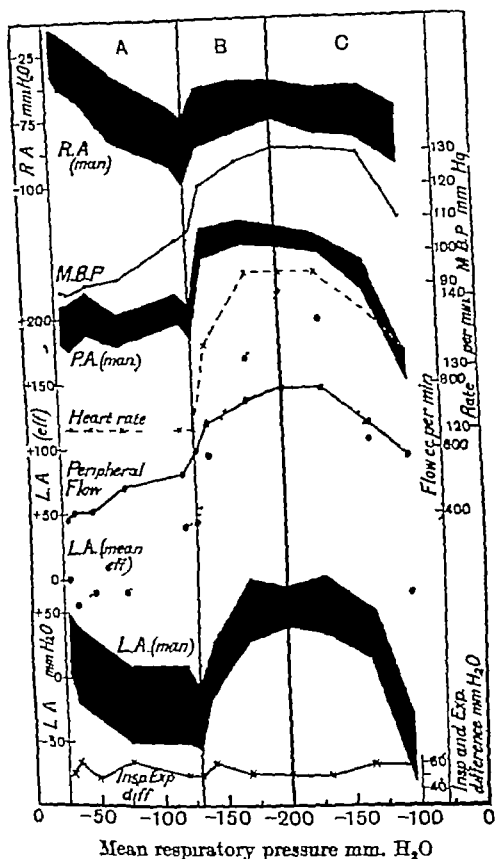
Fig 5 Dog 8 5 kilos Pericardium closed. Respiration rate = 14 per min Effect of reduced mean respiratory pressure accompanied by cardiac acceleration The maximum and minimum pressures in the two auricles and in the pulmonary artery are shown

the preparation suspended in the respiratory chamber For this reason, and owing to the internal structure of the lungs, the mechanism of alveolar expansion and also of changes in the calibre of the blood capillaries, may in our experiments differ slightly from the normal Apart from these considerations, negative pressure ventilation in the manner described, should in the main have the same effects on the circulatory system and on the lung air entry as a reduction of intrathoracic pressure in the whole animal

The experimental results show that a reduction in the mean respiratory pressure produces effects upon the heart-lung preparation which may be ascribed, wholly or in part, to an increased diastolic filling of the heart This increased diastolic filling with outputs of from 300–600 c c per minute does not raise the manometric venous pressure (mean) more than the lowered respiratory pressure reduces it, unless there is a further concomitant increase in cardiac work, such as might occur when the heart is accelerated and the systemic resistance elevated It is not easy to estimate how far these results may apply to the dog under conditions of exercise, since in all probability the heart, owing to nervous influences, rarely reaches the pericardial limit Notwithstanding this, the results do indicate the separate factors which are involved in determining the mean venous pressure when the lungs are ventilated by a negative pressure and when that pressure is made still more negative The part played by the contraction of the skeletal muscles, or by the descent of the diaphragm, in aiding the circulation can be imitated in the preparation by imposing a steady or rhythmic positive pressure on the venous bag K (Fig 1) Both these procedures have the effect of steadily raising, or rhythmically raising and lowering, the venous pressure, and so augmenting the cardiac output, the blood is merely redistributed so that the volume of the external circuit is diminished Data of these experiments are not given, since the quantitative effects obtained by diminishing the capacity of the external circuit have been previously described (3)

SUMMARY

(1) A closed circuit heart-lung preparation with negative pressure pulmonary ventilation is described

(2) In confirmation of Möllgaard's experiments on the whole animal, it is found that a reduction in the mean respiratory (intrathoracic) pressure increases the total output of the heart

(3) Respiratory pressures of − 400 mm H_2O are not injurious to the heart provided it is in good condition

(4) The change in the mean venous pressure produced by lowering the mean respiratory pressure depends upon the extra amount of work which the heart is called upon to perform as the result of the lowered respiratory pressure

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A METHOD OF PROPELLING AIR ROUND SMALL ENCLOSED CIRCUITS   By F M HAINES, Ph D

(From the Botanical Department, East London College)

The apparatus described was originally invented as a substitute for the india-rubber bulb device employed for passing air containing respired CO_2 through the indicator solution in Osterhout's Indicator Method of measuring respiration, but appeared to be capable of wider application

The principal advantages of the mechanism suggested are (1) that the air stream delivered is much more constant than that given by a bulb or by other mercury devices, the delivery being maintained for 4/5 of the cycle instead of only 1/2, (2) the variations in pressure in the circuit are small, (3) india-rubber in the circuit can be dispensed with altogether except for two inches of 1/8 inch tubing for connections, this being an important point in the Osterhout apparatus mentioned since with very slowly respiring material the leak of the rubber bulb to CO_2 even when well varnished with celluloid varnish is apt to become a troublesome source of error, (4) the whole of the air is forced to pass round the circuit, (5) there are no dead spaces in the circuit and (6) the circuit is completely enclosed without possibility of leak to or from the air

The glass piece, *ADDEBC*, (Fig 1) is mounted on a spindle, *S*, placed through a piece of cork, *M*, wedged and cemented between the tubes *C* and *D* at about a quarter of the length from the end   (The method of fixing the mounting-cork and spindle is shown in Fig 2, the small piece of cork, *m*, being inserted last) The spindle, *S*, also carries one end of a metal arm, *L*, the other end of which is attached to a second cork mount, *N*, on the glass piece by a pin, *P*   The whole system is free to move about the spindle, *S*, between the planes marked by dotted lines, *dd* and *rr*, and when in action is repeatedly raised slowly into the "raised" position, *rr*, and then suddenly dropped into the "drop" position, *dd*, the spindle, *S*, being mounted in bearings (not shown), and the shock of the drop being absorbed by a spring attached to the end of *L* (also not shown) which falls upon a stop The oscillations are produced mechanically by a rod, *F*, running in guides, *G*, and actuated by a snail cam, *H* (revolving counter clockwise in the figure), mounted on the same spindle, *O*, as a pinion, *J*, engaging with a worm-wheel, *K*, on the spindle, *Q*, of a 1/16 H P electric motor   (In practice the arrange-

ment can be made very compact by mounting the bearings for the spindles, S and O, and the guides, G, on the same base and running the

Fig 1.

Scale

10 cms

5

0

Fig 2.
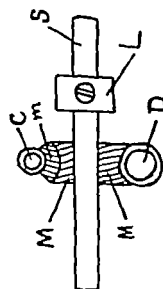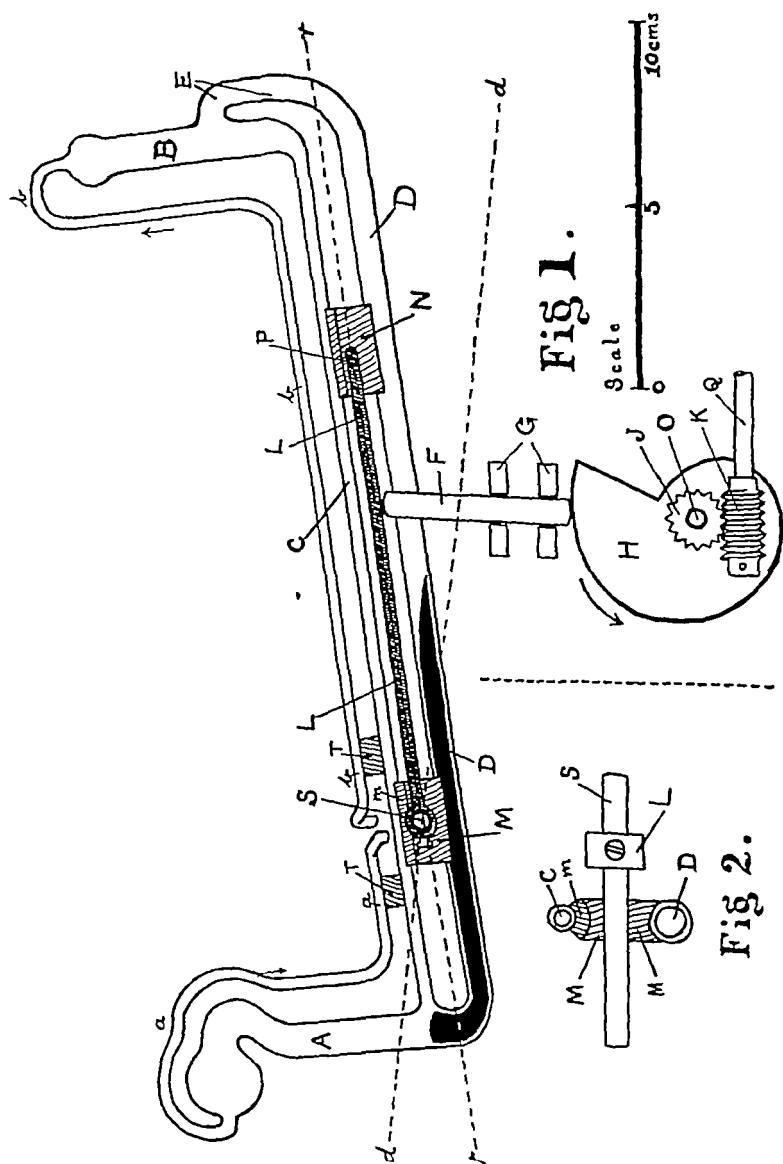
Internal diameters of glass tubings = A and B, 8 mms C, 3 mms D, 0 mms   and aa and bb, 2 mms

spindle, O, in the same plane as the rest of the apparatus instead of at right angles to it as shown in the figure)

Sufficient mercury is introduced into the apparatus to come just up to the level at which C enters A when the glass piece is in the fully raised position, rr, as shown   On the "drop" when F falls on the cam the mercury falls to the right hand end of D, about one-third of it being thrown over the bend at E into B and the rest remaining in D On the gradual rise to the rr position two columns of mercury fall simultaneously in C and D from right to left forcing air before them into A and out the outlet, aa (The vessel A is so shaped as to prevent mercury being splashed over into the outlet tube, aa, during this process) Meanwhile more air is drawn into B, E and D through the inlet, bbb On the subsequent drop all the mercury returns to the right hand end by the lower tube, D, forcing this air into A through the tube C (It will naturally not travel back round the external circuit as the solution through which it is being bubbled forms a trap) The limbs, A and B, are made about 7 cm high and slightly bulbed at the top in order to prevent mercury being splashed into the inlet and outlet tubes The inlet, bbb, and the outlet, aa, are led off, as shown, in such a way as to end one on each side of the apparatus close to the two ends of the spindle, S For rigidity's sake they are supported near the ends by the cork pieces, T, T, cemented to the tube C By this means their (varnished) rubber connections may either cross or lie along the axis of oscillation of the whole system and the amount of rubber necessary is cut down to an absolute minimum

The pressure developed is sufficient to bubble air through a fine nozzle under 6 inches of water, reagent, indicator, etc , the maximum pressure reached with an apparatus of the dimensions given being about 10 inches of water   The snail cam should revolve once in 5 seconds, the air then being expelled for 4/5 of the total time, provided that the amount of mercury and the amplitude of the oscillations are both correctly adjusted   For the purpose of the latter adjustment provision is made in the mounting for the rod, F, to be set at will to operate upon any part of the arm, L, at any desired distance from the axis, S

An apparatus about 30 cm long such as that shown in the figure when used to force a stream of small bubbles through a tube of reagent 6 inches deep delivers 70 c c per minute, but smaller sizes down to a length of 10 or 12 cm work quite satisfactorily when only a smaller delivery is required

In conclusion, I am much indebted to the College Instrument Maker for constructing the mechanical parts

REFERENCE.

Osterhout Journ. Gen. Physiol. I p 17 1918

STUDIES ON THE MOVEMENTS OF THE ALIMENTARY
CANAL   I   The effects of electrolytes on the rhythmical
contractions of the isolated mammalian intestine
By H. E. MAGEE and C. REID

(From the Rowett Research Institute and the Physiological Department,
University of Aberdeen)

WITHIN the last decade the physiology of skeletal muscle has progressed with extraordinary rapidity   In comparison, the physiology of involuntary muscle is in a very backward state   Our lack of knowledge of the fundamental properties of smooth muscle is "particularly regrettable, because in the treatment of disease a knowledge of the properties of cardiac and plain muscle is of far greater importance than a knowledge of the properties of skeletal muscle" [1]

The effects of ions on living tissues have been worked out chiefly on small animals or on cardiac muscle, while intestinal muscle has received scant attention. This seemed to us, therefore, a suitable field for investigation, the need for which Evans[2] emphasises in his review of the literature

This paper deals with the effects of varying the ionic concentration of the environment on the isolated small intestine of the rabbit   Work along other related lines will be reported later

Experimental.

Full-grown rabbits fed on a diet of pasture, cabbage, turnips, carrots, etc , were used   The animals were killed by a blow on the head, a piece of jejunum just distal to the duodenum was rapidly freed from mesenteric attachments, cut out and immersed at once in oxygenated ice-cold Tyrode's solution   This modification of Ringer's solution was used for these experiments because Neukirch[3] found it to be the most suitable for the rabbit's intestine   Its composition is as follows

NaCl	0 8 p c	
KCl	0 02	
CaCl$_2$	0 02	All in anhydrous state
MgCl$_2$	0 01	The water used was
NaH$_2$PO$_4$	0 005	fresh glass distilled
NaHCO$_3$	0 05	
Glucose	0 1	

The *p*H of this solution is 7 6 to 7 8   It was checked in each experiment

A *suitable length* was immersed in Tyrode's solution at 37° in a Burn and Dale bath(4), into which a stream of oxygen bubbles was passed continuously, and the movements recorded on a slow drum

Each ion was studied after the same general plan   The appropriate salt was omitted, and then additions were made gradually until excess was present   Sometimes the concentrations of more than one salt were varied in this way at the same time, but, unless otherwise stated, only one ion was varied, the other constituents being kept constant

The distance between the timing (half minutes) and the foot point of the contractions represents the tone except in Fig 1 *a, b* and *c*, where the intervening paper was excised to economise space

Results

Sodium   Lack of NaCl (Fig 1 *a, b, c* and *d*)   In Tyrode containing 0 2 p c NaCl when the osmotic pressure was not kept normal the beats were abolished and the tone fell, but, when the osmotic pressure was

a	b	c	d
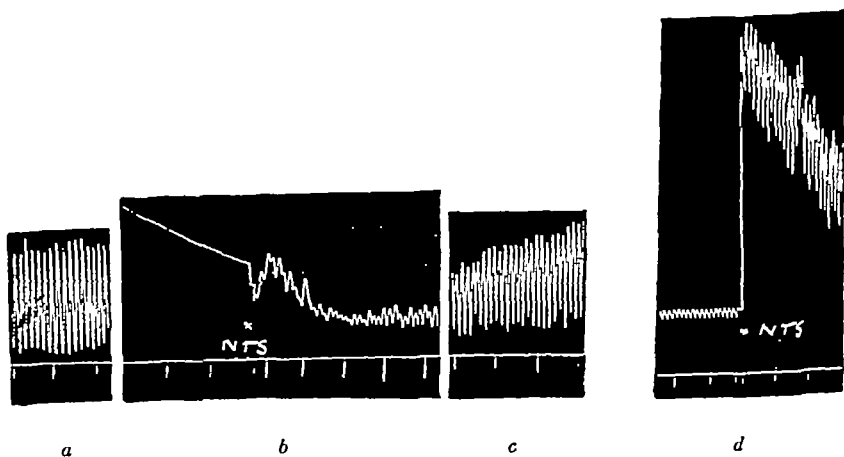
Fig 1   *a* = N T S   *b* = Change from 0 2 p c. NaCl (Os Pr not made up) to N T S   gradual recovery   *c* = After interval of 10 minutes   *d* = Change from 0 2 p c NaCl (Os Pr made up) to N T S   immediate recovery

   Note.   N T S = normal Tyrode s solution.

kept normal by the addition of cane sugar, beats about a quarter the size of the original were *evoked   Change to Tyrode's solution gave* in the former case a slow return of contractions and tone, but in the latter

an immediate return of both   Other experiments showed that when the osmotic pressure was not compensated contractions did not begin until the concentration of NaCl was raised to 0 6 p c and then the amplitude was about three-quarters of the original Further, the response to the addition of more NaCl was delayed The rate was not appreciably affected by low concentrations of NaCl

Excess of NaCl 0 9 p c NaCl raised the amplitude and then lowered it slightly after about 5 minutes Concentrations above this had a similar effect, the tone and rate increasing progressively and the amplitude increasing up to 1 1 p c NaCl and then diminishing gradually Change back to normal Tyrode increased the tone, which fell gradually, the amplitude being somewhat lower than the original at first Complete recovery was established after about 2 hours 1 6 p c NaCl practically abolished the contractions

Calcium Lack of $CaCl_2$ (Fig 2 *a*, *b* and *c*) Complete absence caused rapid fall of tone, slowing, and gradual disappearance of contractions The effect of even small additions was a sudden increase of tone, followed by a more gradual return of amplitude and rate The amplitude and tone were very often greater in concentrations of about 0 01 p c than in normal Tyrode (0 02 p c $CaCl_2$)
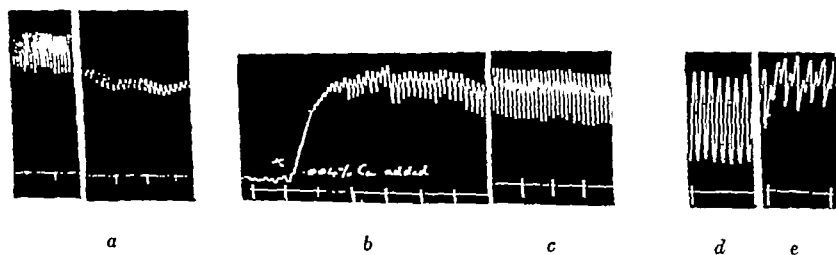
Fig 2 *a* = Showing change from ‰ T S to Ca free fluid From *a* to beginning of *b* 22 minutes interval *b* = At *x*, 0 004 p c $CaCl_2$ added to fluid in bath *c* = $CaCl_2$ increased to 0 008 p c   *d* = ‰ T S *e* = $CaCl_2$ has been increased to 0 08 p c

Note a b, c, from the same piece of intestine d, e, from another piece

Excess of $CaCl_2$ (Fig 2 *d* and *e*) Additions up to 0 04 p c lowered the amplitude and increased the rate Further additions increased tone and rate but lowered the amplitude progressively In large excess the contractions were irregular owing to incomplete relaxation. These effects were reversible

Potassium Lack of KCl (Fig 3 *a*, *b*, *c* and *d*) Complete absence caused an immediate increase in tone, which fell slightly later, and a

very small beat, which persisted   Additions of KCl up to 0 02 p c,
diminished tone and amplitude immediately and only after several
minutes were the tone, amplitude and rate restored   The longer the
exposure to low concentrations of KCl, the slower was the recovery of
the amplitude   These findings confirm Clark's[5] on the small intestine
of the rabbit

a   b   c   d   e
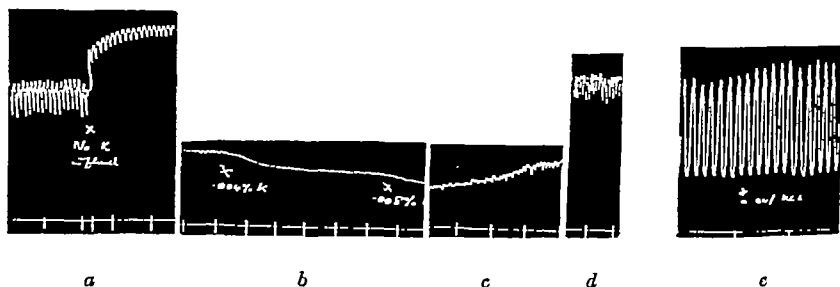
Fig 3 a=Change at x from N T S to K free fluid. a to b=20 minutes interval b=At
each x 0 004 p c KCl added. c=KCl increased to 0 016 p c KCl six minutes
previously c to d=15 minutes' interval e=From a separate piece of intestine,
KCl in bathing fluid increased from 0 02 p c. to 0 04 p c at x

Excess of KCl (Fig 3 e)   Concentrations of KCl between 0 02 and
0 06 p c increased the amplitude and slowed the rate, the tone being
scarcely affected   Concentrations above 0 06 p c increased the tone and
diminished the amplitude and rate   The effects of K in excess were
reversible

The ratio $\frac{cCa}{cNa+cK}$ Clark[6] has shown that alterations in the
ratio, whether due to increase or decrease of any of the three ions, pro
duce definite results on the frog's heart   We have already referred to
the effects of varying the concentration of one ion at a time   The results
of lowering and raising the ratio by simultaneous variations in two of
the factors in some cases merely corroborated the above results and in
others showed nothing of a definite nature

Hydrogen ion (Fig 4)   The pH was varied by withholding and then
adding NaHCO$_3$ to the bathing fluid   As the pH was raised from 5 6
to over 9 (a) the rate increased up to pH 8, and then began to fall,
(b) the amplitude (very irregular in acid media) became regular and
increased until pH 8 to 8 4 and then declined, (c) the tone (very low in
acid media) rose to a maximum at pH 7 2 to 8, and then began to fall
These results agree with those of Evans and Underhill[7] and of
Hammett[8] on the small intestine of the rabbit and rat respectively,

and are comparable to those of Kupaloff(9,10) on the frog's stomach after intravenous injection of alkali and acid respectively

pH $= 6$ pH $= 7$ pH $= 7$ 8 pH $=$ over 8

Fig 4.

Magnesium (Fig 5) When $MgCl_2$ was added to a Mg-free fluid immediate effects were produced the beats became more regular, the rate slower and the tone lower When added in excess the amplitude, rate and tone fell. Even large excess (0 08 p c) did not have a very marked effect on the beats The effects of adding Mg in excess were reversible

PO₄ Only the influence of excess PO_4 was studied because the withdrawal of NaH_2PO_4 would change the pH A mixture of solutions of $M/15$ Na_2HPO_4 and $M/15$ KH_2PO_4 in amounts necessary to give a pH of 7 (Cullen(11)) was made and added to the bathing fluid This was made up without KCl because when 1 c c of the phosphate mixture was added to the 125 c c of fluid the cK was just about the same as in Tyrode's solution The slight excess of Na in the fluid was not considered of any great importance 1 c c phosphate mixture raised the tone and lowered the amplitude and rate immediately 2 c c lowered the tone and amplitude a little These effects were reversible Further increases were complicated by the action of the unavoidable excess of Na and K
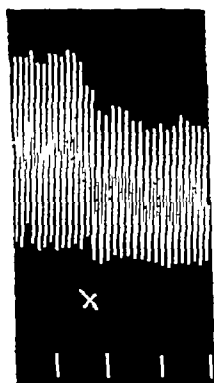
Fig 5 At x change from Mg free fluid to one containing 0·01 p c $MgCl_2$

Iodine (Fig 6) Iodine is not a constituent of any physiological saline solutions, but its effect on the intestine is of interest owing to its being present in plants, milk and blood in small amounts (Leitch and Henderson(12)) Its effect on the intestine was studied by replacing normal Tyrode's solution by equal volumes of a similar solution containing an equivalent amount of NaI instead of NaCl Replacements up to about 15 c c (0 26 p c NaI) improved the amplitude progressively Larger replacements lowered the amplitude but increased the rate by

three or four per minute These effects persisted after several changes
of Tyrode's solution   Finckh(13) found that complete replacement of
NaCl by NaI had a toxic effect on the frog's heart   Unfortunately
his replacement was immediately complete, not gradual, so that exact
comparison with our experiments is not possible   On the other hand,

                    a   b   c
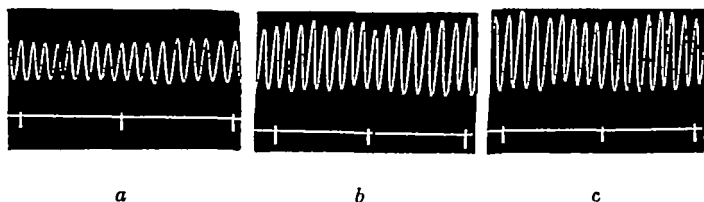
Fig 6   a = N T S   b and c = After replacing 2 and 8 c c respectively of N T S by equal
volumes of similar fluid containing equivalent amounts of NaI for NaCl

Thunberg(14) showed that low concentrations of KI had, in some experi
ments, a stimulating action on the O_2 consumption of frog's skeletal
muscle   Perhaps a like stimulating
effect may be operative also in the
case of plain muscle

Manganese (Fig 7) The biological
importance of manganese may be in-
ferred from the fact that it is a con-
stituent of many animal tissues and
plants (Reiman and Minot(15,16),
McHargue(17)) It has also recently
sprung into considerable favour with
many clinicians as a therapeutic
agent (Nott(18))

    Small amounts of a 1 p c solution
of $MnCl_2$ were added to the bathing
fluid   Very small additions, 0 1 c c
(giving a concentration of 0 0008 p c),
increased the tone and amplitude
Larger additions, 0 3 c c , lowered
tone, amplitude and rate   0 5 c c
almost obliterated the contractions
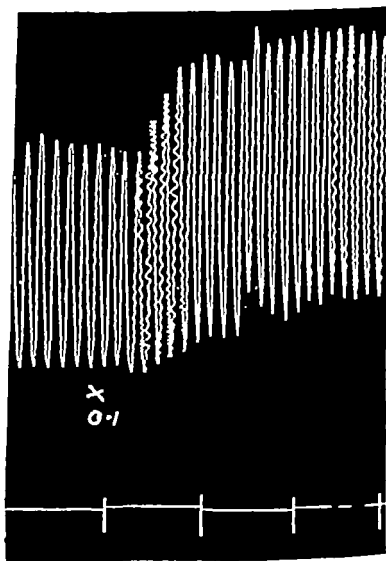
Fig 7   At x 0 1 c c of 1 p c MnCl$_2$
added to 125 c c N T S

Discussion

It is clear from the results that the osmotic and specific effects of Na are closely associated Apparently a certain minimum osmotic pressure is necessary as a basis for the operation of the specific effect of Na If the osmotic pressure has been lowered some time is required for its re-establishment after the addition of the normal amount of NaCl Very probably the mechanism is one of simple diffusion, for, if it were a case of combination with cell constituents in the sense put forward by Loeb[19] for skeletal muscle, cane sugar could not have taken the place of NaCl so well. The height of contraction is apparently directly proportional to the cNa While Na in the presence of a proper osmotic pressure and of Ca and K can initiate contractions, the actual pace seems to be a function of the Ca/K ratio Ca progressively increased the rate while K consistently decreased it Eichholtz and Starling[20] have distinguished two modes of action of ions on the kidney cells—an immediate or ionic action and a delayed action or one the result of chemical union. Our results permit of analogous differentiations Ca in all concentrations, and Na and K in super-optimal concentrations, would fall into the first category, since the effects of further additions were immediate When K was added to sub-optimal concentrations of this ion, an immediate effect was obtained, followed after a long interval by gradual recovery Thus K in low concentrations appears to have a twofold action—an ionic action and a combining action—the former, apparently, being preliminary to the combination of the ion with the cell constituents It is significant that Clark[5] found a loss of K by the frog s heart as a result of perfusion with K-free Ringer The mode of action of Na in sub-optimal concentrations is complicated by the concomitant variation in osmotic pressure, but, when this is kept constant, its action seems to be of an ionic nature

The immediate effect of adding Ca to fluids low in this ion was a marked increase of tone followed by gradual improvement in amplitude and in rate It therefore looks as if the recovery of tone was in some way related to the restoration of normal contractility, thus suggesting some support for Höber's[21] view that the physiological action of Ca is to be interpreted in the light of its consolidating action on colloidal systems The work of Widmark[22, 23] and of Chambers and Reznikoff[24] support this opinion The former showed that low concentrations of $CaCl_2$ caused different kinds of muscle to shrink. Chambers and Reznikoff found that the cell membrane of living amœbæ is easily

permeable to NaCl and KCl but not to $CaCl_2$, and that, while the former salts disintegrate the cell membrane, the latter consolidates it Clark's[6] work on the frog's heart has shown that the physiological action of Ca is brought about through union with lipoid substances, in which way the semi-permeability of the cell membrane is maintained Wieland's[25] results on surviving pieces of rabbit's intestine are in harmony with Clark's It is, therefore, to be inferred that the effect of adding Ca salts to fluids low in Ca is to restore the semi-permeability of the cell membrane a condition which appears to be related in some way to the contractile process

We can distinguish three zones of action of K in regard to concentrations In concentrations of KCl up to 0 02 p c it was completely antagonistic to Ca Between 0 02 and 0 06 p c the rate was slowed, but the amplitude and sometimes the tone were stimulated To this extent, therefore, our results agree with Höber's[26] contention that mammalian intestinal muscle is relatively insensitive to the depressant action of K Concentrations above 0 06 p c increased the tone but lowered the amplitude and rate It is of interest to note that Kupaloff[27] found that, in the frog, intravenous injections of KCl produced a rise of tone of the stomach and $CaCl_2$ strong spastic contractions

There is a very extensive literature dealing with the influence of electrolytes on cardiac and skeletal muscle, but it is evident from the data presented here that mammalian intestinal muscle does not react to changes in the fluid in which it is bathed in the same way as cardiac or skeletal muscle Intestinal muscle must obviously be the subject of special study, and further experimental data will need to be accumulated before the fundamental differences between the physiology of mammalian intestinal muscle and skeletal muscle of cold blooded animals can be profitably discussed.

SUMMARY

The response of the isolated surviving jejunum of the rabbit to changes in ionic environment was studied

(1) The osmotic and specific effects of sodium were closely associated A certain osmotic pressure was necessary before the specific effects of sodium could operate Within limits, the amplitude of contraction was directly proportional to the concentration of sodium Excess of sodium increased the tone and diminished the amplitude

(2) The rate varied directly as the ratio Ca/K Calcium progressively

increased the tone The amplitude was at a maximum in a concentration of calcium of about half that in Tyrode's solution.

(3) Three concentration zones of action of potassium were distinguished with 0–0 02 p c KCl, tone was lowered and contractions abolished These recovered after several minutes' delay With 0 02–0 06 p c KCl, the amplitude was augmented, the rate slowed and the tone generally unaffected

Above 0 06 p c KCl, tone was increased and amplitude and rate diminished.

(4) Calcium almost completely antagonised potassium. The effects of additions of calcium to fluids of all concentrations of this ion, and of additions of sodium and potassium to fluids, of optimal osmotic pressure in the one case, and of optimal potassium content in the other, were immediate. The effects of additions of sodium to fluids of sub-optimal osmotic pressure, and the ultimate effects of additions of potassium to fluids of sub-optimal potassium content were delayed. The probable significance of these results is discussed

(5) The optimal cH zone was pH 7 to pH 8 Below pH 7 tone and rate were lowered and contractions were very irregular Above pH 8 tone and rate were distinctly, and amplitude slightly, lowered.

(6) Magnesium, although not absolutely essential for contractility, appeared to have a regularising influence on the contractions

(7) The phosphate ion in excess stimulated tone and lowered the amplitude and rate

(8) Iodine in low concentrations (up to 0 26 p c NaI) increased the amplitude and in higher increased tone and rate but diminished the amplitude These effects came on gradually and persisted.

(9) Low concentrations of manganese (0 0008 p c MnCl$_2$) temporarily stimulated contractions higher concentrations (0 002 p c MnCl$_2$) depressed them.

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THE LACTIC ACID MAXIMUM OF CARDIAC MUSCLE

By DOROTHY ARNING

(From the Physiological Department, Manchester University)

THE following experiments were carried out as a first step in the investigation of the chemical changes taking place during the contraction of cardiac muscle and consist in comparative estimations of the lactic acid maximum of cardiac and skeletal muscle The investigation has since been carried out in further detail by Hines, Katz and Long(1), using mammalian muscle, and their results are in good agreement with mine

Estimation of Lactic Acid

Before starting the experiments, the method by which the lactic acid was to be estimated had to be chosen and investigated It was obvious that the amount of material available would necessitate the use of a micro-method, and the ones considered were those of Meyerhof(2,3) and of Clausen(4)

In the first place, the technique of the permanganate oxidation process of Clausen was compared with that of Meyerhof, using a solution of zinc lactate Clausen's method was found to give excellent results both as regards the actual percentage recovery of lactic acid and its variation. In Meyerhof's method the percentage recovery was smaller and showed a much greater variation from one experiment to another Moreover, Clausen's method presented fewer difficulties of manipulation than that of Meyerhof and afforded a considerable saving in time The percentage recovery was of the same order of magnitude as that found by Clausen himself, that is, from 92–95 p c when using from 2 5–4 0 mg of lactic acid It was therefore decided to carry out the process of extraction from muscle and purification according to Meyerhof's technique, with the introduction of certain modifications, and to carry out the oxidation, distillation and titration according to Clausen

The main steps of the process were as follows the muscle was ground in a mortar with alcohol and powdered glass, the mixture was transferred to a beaker and allowed to stand over night It was filtered first

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mended by several workers as being the most suitable salt of lactic acid for control estimations and, as already stated, gave excellent results on the oxidation process alone It was ultimately realised that these inconsistencies must be due to the small solubility of zinc lactate, which, in certain cases, prevented its being completely re-dissolved when the residue from the evaporation of the original alcoholic solution was treated with saturated ammonium sulphate solution There is, at this stage of the process, a considerable deposit of ammonium sulphate on the dish, which would readily obscure particles of zinc lactate

Control estimations were repeated, using lithium lactate, and quite consistent results were obtained (Table I)

TABLE I. Estimation of Lithium Lactate Solution. Complete Process
5 c.c. solution = 8 95 mg lactic acid.

No	Amount taken mg. HL	Amount estimated mg HL	Percentage loss
1	8 95	7 87	12 0
2	8 95	7 59	15·2
3	8 95	7 27	18 8
4	8 95	7 65	14 5

Mean percentage loss = 15 1

The percentage recovery of the complete process was found to be 85 p c

The animals used for the comparative estimations on cardiac and skeletal muscle were the frog and the tortoise The weight of the heart of an ordinary frog is less than 0 1 grm , but it was hoped that by using the *Rana esculenta* (variety *ridibunda*) obtained from Hungary, which is many times larger than the ordinary frog, estimations could be carried out on two or three hearts Unfortunately, the heart was found to be small in comparison with the rest of the body, so that to obtain results of any accuracy a large number had to be used

As the supply of these frogs was spasmodic, experiments were also carried out on the tortoise, which has a heart sufficiently large for estimations to be made on one only

Method

Frog The ventricle only was used It was cut longitudinally with scissors and the two pieces pressed between filter paper to free them from blood The muscle from several hearts was weighed in a weighing bottle Cotton-wool soaked in chloroform was inserted between the stopper and the bottle and the whole was placed in an oven at 40° C for an hour

through muslin and then through paper and the muscle residue extracted with fresh alcohol, twice for amounts of muscle under 1 5 grm and three times for amounts over 1 5 grm. These washings were evaporated on a water bath and the original filtrate was added last and the whole taken down to 1–2 c c , this procedure reduced to a minimum the decom position of lactic acid by heat The residue, which had usually evaporated to dryness on standing, was extracted four times with 2–3 c c of satu rated ammonium sulphate solution and filtered at the pump through a small asbestos filter, which was connected by an adapter to a separating funnel The adapter was rinsed with 2 c c of 4 p c caustic soda and the volume made up to 15 c c with distilled water The solution was extracted three times with 2 c c of benzene, to remove traces of alcohol, the upper benzene layer being removed by a pipette attached to the pump The last traces of benzene were removed by evaporation under reduced pres sure, by connecting the separating funnel to the pump This was con sidered an improvement on the method used by Meyerhof of again evaporating on a water bath, during which there is always a risk of decomposing some lactic acid

The solution was then roughly neutralised by the addition of 2 c c of 0 1 N sulphuric acid and then, according to the amount of lactic acid present, either transferred direct to the oxidation apparatus or else made up to 50 c c , of which two or three aliquot parts of 10 c c were used for oxidation. The latter procedure was adopted for two reasons, in the first place it was thought that there would be a smaller relative loss in the intermediate processes of the estimation if a larger quantity of lactic acid were used, whilst in the oxidation process, perfectly con sistent results had been obtained with less than 2 mg of lactic acid, which is the amount that can be most rapidly and most efficiently dealt with In nearly every case very close agreement (i e to within 0 1 mg lactic acid) was obtained between the two estimations and when this did not occur a third aliquot part was used

The oxidation was carried out in all details according to the method described by Clausen. The end-point of the titration was always found to be perfectly clear-cut and the difficulties of lack of permanence, mentioned by Clausen and also experienced by Long(5), did not occur

Controls on the complete process were carried out on zinc lactate solution and on muscle in rigor mortis with and without added zinc lactate Whilst estimations on two or more portions of the same muscle gave perfectly consistent results, those on zinc lactate and on muscle with added zinc lactate showed wide variation. Zinc lactate is recom-

mended by several workers as being the most suitable salt of lactic acid for control estimations and, as already stated, gave excellent results on the oxidation process alone It was ultimately realised that these inconsistencies must be due to the small solubility of zinc lactate, which, in certain cases, prevented its being completely re-dissolved when the residue from the evaporation of the original alcoholic solution was treated with saturated ammonium sulphate solution There is, at this stage of the process, a considerable deposit of ammonium sulphate on the dish, which would readily obscure particles of zinc lactate

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The percentage recovery of the complete process was found to be 85 p c

The animals used for the comparative estimations on cardiac and skeletal muscle were the frog and the tortoise The weight of the heart of an ordinary frog is less than 0 1 grm , but it was hoped that by using the *Rana esculenta* (variety *ridibunda*) obtained from Hungary, which is many times larger than the ordinary frog, estimations could be carried out on two or three hearts Unfortunately, the heart was found to be small in comparison with the rest of the body, so that to obtain results of any accuracy a large number had to be used

As the supply of these frogs was spasmodic, experiments were also carried out on the tortoise, which has a heart sufficiently large for estimations to be made on one only

Method

Frog The ventricle only was used It was cut longitudinally with scissors and the two pieces pressed between filter paper to free them from blood The muscle from several hearts was weighed in a weighing bottle Cotton-wool soaked in chloroform was inserted between the stopper and the bottle and the whole was placed in an oven at 40° C for an hour

The hearts, in any one experiment, were all taken from the same batch of frogs

The skeletal muscle was taken from one of these frogs and consisted of either one gastrocnemius or several leg muscles

Tortoise The ventricle was cut into several pieces, pressed between filter paper and put into rigor as above

The skeletal muscle was taken from the legs and from inside the shell. It was found difficult to get a representative sample, since it showed noticeable variation in composition, according to the site from which it was taken.

Results

In calculating the true lactic acid value from that estimated, the average loss is taken to be that of the lithium lactate controls, that is, 15 p c

The preliminary experiments on cardiac muscle of both frog and tortoise (Tables II A and III A) were made before one or two of the

TABLE II. Lactic Acid Maximum. Cardiac and Skeletal Muscle.

FROG

		Cardiac					Skeletal		
No	No of hearts	Muscle grm.	HL esti mated mg	True HL value mg	Per centage HL in muscle	No	Muscle grm.	HL esti mated mg	True HL value mg	Per centage HL in muscle

A. (Frog arrived 29 vııı 22)

No	No of hearts	Muscle grm.	HL esti mated mg	True HL value mg	Per centage HL in muscle	No	Muscle grm.	HL esti mated mg	True HL value mg	Per centage HL in muscle
1	3	0 629	0 78	0 92	0 146	—	—	—	—	—
2	4	1 006	1 22	1 44	0 143	—	—	—	—	—

Mean percentage HL=0 145

B (Frogs arrived 10 v 23)

| 3 | 8 | 1 281 | 1 85 | 2 18 | 0 169 | 8 | 2 572 | 6 24 | 7 34 | 0·285 |
| 4 | 6 | 1 160 | 1 80 | 2 12 | 0 182 | 9 | 2 603 | 8 23 | 9 68 | 0·372 |

Mean percentage HL=0 175   Mean percentage HL=0 328

Ratio—cardiac   skeletal=0 53

C. (Frogs arrived 30 vııı 23)

5	20	3 526	7 13	8 40	0 238	10	1 404	6 46	7 60	0 541
6	14	1 993	5 51	6 48	0 325	11	1 638	7 54	8 87	0 541
7	15	1 818	4 38	5 16	0 284	12	1 387	4 67	5 49	0·396
—	—	—	—	—	—	13	2 066	9 56	11 26	0 545

Mean percentage HL=0 282   Mean percentage HL=0 506

Ratio—cardiac   skeletal=0 56

later improvements in technique had been introduced   All the other estimations were carried out according to the final routine, aliquot parts were taken for oxidation except in the case of the determination on

TABLE III. Lactic Acid Maximum. Cardic and Skeletal Muscle.

Tortoise.

A.		Cardiac						Skeletal			
Date	No of hearts	Muscle grm.	HL esti mated mg	True HL value mg	Per- centage HL in muscle	No	Date	Muscle grm.	HL esti mated mg	True HL value mg	Per- centage HL in muscle
Jan. 1923	1	1 157	2 40	2 82	0 240						
"	1	0·58	1 49	1 75	0·303						
"	1	0·915	1 90	2 24	0 244						
Feb 1923	1	0 644	0·645	0·76	0 118						
"	1	0·75	2 42	2 85	0·379						
			Mean percentage HL=0·257								
B											
Feb. 1923	4	3 715	8 55	10·06	0 270	10	Feb 1923	2 550	6·12	7 19	0·262
						11	"	2·060	9 22	10·84	0 514
Apr. 1923	4	3 641	9·05	10·65	0 293	12	Apr 1923	2 946	8·07	9·50	0 322
						13	"	3·056	9·65	11 35	0 372
Sept. 1923	1	1·409	3 54	4·17	0 295	14	Sept. 1923	1 902	7 39	8 70	0 464
"	1	1 537	3 44	4·05	0·263						
			Mean percentage HL=0·280						Mean percentage HL=0·391		

Ratio—cardiac skeletal=0 72.

cardiac muscle of Tables II A and B and III A where the amount of material used was not large enough for division.

Determinations on cardiac and skeletal muscle of the same animal are tabulated on the same line In Table III B, estimations 10 and 11 are on skeletal muscle of the same tortoise, as also estimation 12 and 13

The lactic acid maximum of frogs' cardiac muscle is in every case lower than that of the corresponding skeletal muscle Estimations on different batches of frogs are entered in separate tables, the values for the two maxima show a fair agreement with one another within each batch, but those of the two series differ from each other by a marked seasonal variation.

The results may be best expressed as the ratio of the cardiac mean percentage to the skeletal mean percentage, which is 0 53 and 0 56 respectively

The lactic acid maximum of tortoise's skeletal muscle shows consider-able variation from one estimation to another, even when two deter-minations are made on the muscle of one animal (cf. Nos 10 and 11) These variations can only be explained as being due to differences in the composition of the muscle, that from the legs often contained much connective tissue so that it was not always possible to obtain a sample

consisting only of muscle fibres The values for cardiac muscle are more consistent and, taking the mean of both, the cardiac maximum is seen to be definitely lower than the skeletal maximum, though the difference is less marked than it is in the case of the frog, the ratio of the cardiac mean percentage to the skeletal mean percentage being as much as 0 72

The theoretical deductions from these results are the same as those put forward by Hines, Katz and Long

SUMMARY

Comparative estimations of the lactic acid maximum of cardiac and skeletal muscle show that in every case, the cardiac maximum is lower than the skeletal maximum

For the frog, the average ratio of the lactic acid maximum of cardiac muscle to that of skeletal was found to be 0 5, whilst for the tortoise it was found to be 0 7

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FATIGUE, RETENTION OF ACTION CURRENT AND RECOVERY IN CRUSTACEAN NERVE

By A LEVIN[1]

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THE study of fatigue in nerve has been hitherto confined chiefly to medullated nerve, non-medullated nerve, judging by the literature on the subject, having received comparatively little attention in this connection

Miss Sowton[1] observed in the olfactory nerve of the pike a gradual decrease in size of consecutive action currents after repeated stimulation The investigation of S Garten[2] on the same subject was conducted with the view of ascertaining whether (as had already been found by Bernstein on medullated nerve) this decrease in size of action currents could not be explained by a purely local decrease in the excitability of the nerve in the immediate proximity of the stimulating electrodes, as distinguished from fatigue conducted along the length of the nerve The arrangement used by Garten to control this consisted of two pairs of stimulating electrodes, of which one, more distant from the lead-off electrodes, was used for prolonged fatiguing stimulation, and the other, nearer the lead-off electrodes, was used for applying short test stimuli only By this means Garten established the existence of "conduction" fatigue, and of recovery also, in the olfactory nerve of the pike

The evidence as to the fatigability of molluscan nerve is not unanimous, and whilst Boruttau[3] compares an octopus nerve in this respect to the frog s sciatic, Burian[4] finds in the octopus and eledone nerves a high degree of fatigability Generally speaking, as Garten (*l c*) sums up in his monograph, the various non-medullated nerves differ very widely from each other in their properties, one property he finds common in all of them, namely, the comparative slowness of their electric manifestations

If, thus, the evidence existent up to the present time as to the

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fatigability of non-medullated nerve is not very extensive and generally little known, still it points quite definitely in the direction of its being much greater than the fatigability of medullated nerve and much more easily observed

The medullated nerves are essentially *fatigue-resistant*, and in connection with this their metabolism is also very small Only recently, by extreme refinement of technique, Prof A V Hill, in collaboration with A C Downing and R W Gerard(5), succeeded in measuring the very small amount of heat produced by the frog's nerve in activity[1]

Being interested in the question of the possible superiority of some nerves over others for the study of metabolism and heat production, Prof A V Hill suggested that I should investigate the limb-nerve of the spider crab (*Maia squinado*) in this connection I have subsequently also tried the nerves of the edible crab (*Cancer*) and of the lobster (*Homarus*) They all gave substantially the same results, except that the lobster nerves survived less well

Method The leg of a large specimen is severed near the body (preferably in the arthrotomic seam) and the shell on the lower side of the leg (in reference to the animal) is removed with the aid of bone forceps to the extent of one short and one long proximal link A few transverse cuts with a hacksaw are helpful The adductor group of muscles is then removed with their tendon and the nerve appears lying upon the abductor group of muscles as a semi-transparent bundle This bundle is composed of many secondary bundles, of thread-like appearance, quite loosely connected together, there being practically no connective tissue Some of these threads are given off to the nearest muscle groups, but it is easy to isolate a bundle going to the next link of the limb consisting chiefly of straight uncut fibres In this manner it is easy to obtain stretches of nerve up to 10 cm long in large specimens of *Maia* In both crabs (*Maia* and *Cancer*) the fore claw leg was not used in the lobster on the contrary only the claw leg was found suitable

The nerves were kept in a moist chamber, either in oxygen or in air, and were stimulated by a Harvard-Porter coil, with the hammer vibrating at a frequency of about 60 per second Single shocks also were used The strength of the stimulus was usually well over maximal The make shocks at this strength were, however, almost ineffective, as shown by tests with single shocks and also by string galvanometer records The stimulating electrodes were of silver

Silver-silver-chloride electrodes, applied to the nerve with filter

[1] See also R W Gerard (19)

paper soaked in sea water interposed, were used at first as galvanometer circuit electrodes, but they proved unsatisfactory They were never strictly non-polarisable (either in wire or foil form) and were also apt to develop large electrode potentials, unless special measures were taken, which made their use inconvenient A slight contamination with fatty matter, which is difficult to avoid, seems to affect their functioning

In the experiments on fatigue, in which the size of the electric response is the indicator, these details are of great importance, fatigue and recovery being easily simulated by the presence of polarisation at the electrodes, the response decreasing with repeated stimuli and increasing again after rest

Typical experiment on polarisation of silver-silver-chloride electrodes A thread soaked in Ringer s solution was bridged across freshly coated electrodes in place of a nerve, and a small current was passed through the circuit at intervals The deflection was read on the scale of a moving magnet galvanometer, and was comparable in size with an action current of a frog s sciatic. The current was always closed for 20 seconds and different intervals of 'rest were allowed. In the following table the upper line indicates the intervals between successive observations, so that for ½ minute interval there is 20 seconds stimulus and 10 seconds rest and so on.

Interval in mins.	+	½	½	½	½	½	½	½	½	½	2	2	2	2	3
Deflection in cm.	17	15	14	12	10	10	10	10	10	10	13	14	15	15	15

We see a steady decrease in size of the deflection with frequent stimuli until a steady state is reached then, with longer periods of rest there is a gradual recovery until a new steady state is reached

Polarisation at the electrodes, however, can never simulate fatigue in every detail and can be easily detected as such.

Zinc-zinc-sulphate electrodes of the gelatine type were found more reliable for experiments of this type and were exclusively used in the majority of the experiments

A moving magnet galvanometer, constructed by Mr A C Downing, was used in most experiments It possessed a very high figure of merit and a deflection time, at the sensitivity used, when slightly under-damped, of about one second The excellent properties of this instrument were of great assistance in the investigation It was employed at a distance of 3 m with a sensitivity of $1 \text{ mm} = 3 \ 3 \times 10^{-9}$ amp The lead-off to the galvanometer was usually monophasic (injured-uninjured) The injury current was compensated by the usual potentiometer method The injury was produced by means of crushing the end of the nerve to the extent of one or two mm.

Results

(a) *General observations* Sea water proved satisfactory as a medium for preserving the nerves and making up the gelatine electrodes The

nerves would keep alive and in good condition in sea water for many hours, and would even recover in it after exhaustion by previous stimulation[1] In a moist chamber the nerves would keep alive for 24 hours and still give strong action currents

The electromotive forces found in these nerves are considerable The injury current in a *Cancer* nerve in one case reached 27 millivolts, it was less (10 to 13 mv) in the spider crab These values were observed at the beginning of an experiment, the injury current subsiding first quickly and then more slowly during its course In the *Cancer* nerve, however, quite often the injury current would first increase and then start to decrease The deflection produced in a long period galvanometer by tetanic stimulation is from 10–20 times greater than from the frog's sciatic How far this is due to a longer duration of each response and how far to a greater maximum E M F was not investigated

(*b*) *Fatigue, retention of action current and recovery* The results of a typical experiment are represented in Fig 1 This diagram has been
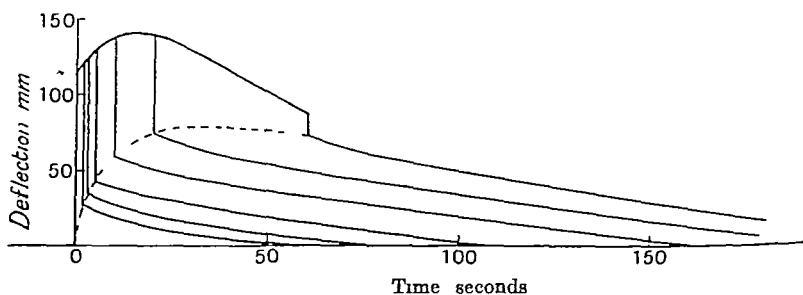
Fig 1 *Action current of spider crab nerve.* Moving magnet galvanometer, monophasic lead, tetanic stimulus Composite diagram, the first vertical line representing for each curve the up swing of the galvanometer in response to the stimulus, the other vertical lines being back swings of the galvanometer at the end of stimuli of 2, 3, 5, 10, 20 and 60 seconds' duration respectively ' Fatigue" is represented by the back swings progressively decreasing in size, ' retention of action current by the galvanometer not returning immediately back to the original line after the stimulus ended

made by observing the movements of the spot of light on the scale and plotting them against time with the aid of a stop-watch It is a composite diagram, the individual curves of which were obtained in the course of a single experiment on a nerve with different durations of stimulus, and superimposed so as to coincide at the beginning of the stimulus Different intervals of rest were allowed between the individual

[1] Hogben (6) found that the heart of *Maia* survives better in a medium containing less magnesium and some urea and it would be of interest to try this also on the nerve

stimulations, the criterion of recovery in each case being the return of the spot of light to the original base-line, the reason for which will be explained later The various points of this group of curves fitted into a consistent whole (within the limits of error of observation, the galvanometer deflection on the scale being read to 5 mm) This in itself shows that the nerve was in good condition and that the criterion of recovery was correct The nerve was kept in oxygen.

If we now start our analysis of the diagram by following up the curve pertaining to the longest stimulus, of one minute's duration, we see that, after swinging up to about 120 mm in response to a stimulus, the galvanometer proceeds to rise to 140 mm. and then starts to decline When, at the end of one minute stimulation, the stimulus is stopped, a surprisingly small back-swing is observed (15 mm only, *i e* from 90 to 75 mm.), the galvanometer then creeping back only slowly towards the original zero The course of events in the case of a shorter stimulus is represented by the other vertical lines, corresponding to back-swings of the galvanometer after 2, 3, 5, 10 and 20 secs stimulus duration The creep of the galvanometer to the original line after each stimulus is also seen.

The first fundamental fact revealed by this experiment is the progressive decline in size of the back-swing of the galvanometer with longer stimuli. Had the stimulus been continued for, say, 2 minutes, no back-swing at all would have been observed on the slowly receding line of the curve at the moment of cessation of the stimulus

In order to assure that the size of the deflection of the moving magnet galvanometer, which shows only the time integral of the individual action currents, is a true measure of the electric response of nerve, and that we have to deal really with the decrease in size of the single action currents and not, for instance, with a gradual transformation of a monophasic response into a diphasic, a few records were taken with a string galvanometer, both with a monophasic, and with a diphasic, lead These have all confirmed the existence of this decrease The stimulus was still maximal at the end of the stimulation, as shown by the fact that a moderate increase of its strength produced no increase in the size of the response

Returning now to the experiment of Fig 1, the second fundamental fact shown by it is that after the stimulus is ended the galvanometer does not return immediately to zero but lags behind and starts slowly to creep back This phenomenon which, under different names, has been described in many excitable tissues (see Discussion below), and for which

the name "retention of action current" seems the most adequate, represents a state of negativity like that associated with activity, but of a more persistent nature than the action currents themselves   In our case the phenomenon is very conspicuous   Referring again to Fig 1 we see that the retention at first increases with the duration of the stimulus, but soon reaches a maximum   Were the nerve stimulated beyond the limit of 1 minute (not shown in the diagram), the retention would start to decline   At this stage, however, the stimulus itself would become ineffective

This retention of action current is a universal phenomenon in crab nerve   It is observed after all kinds of stimuli and even after just liminal single shocks, so that it cannot be due to injury by excessive stimulation   It is evidently intimately connected with the action current itself, and not, for instance, with the stimulating current   This is shown by the fact that the effect disappears when the stimulus is reduced to a just subliminal strength, and it does not increase indefinitely when the stimulus is increased beyond the maximal strength, neither does the reversal of the direction of the stimulating current produce any effect

The evidence, collected under (*d*), shows directly that the retention is due to an increase of the negativity along the nerve trunk, and not to a decrease of the negativity of the injured end

The time taken by the retention to wear off varies with the duration of the stimulus and the condition of the nerve   It is 4 to 10 minutes after a prolonged tetanic stimulus and only a few seconds after single shocks   The better the condition of the nerve the quicker the retention wears off, and in poor preparations the galvanometer may never come back at all

Another fact is to be recorded, although it is not so constant in its appearance as the two described above   The total deflection of the galvanometer, as made up (*a*) by the amount of the retention present at any given time, and (*b*) by the action current proper, tends to be a constant value   In other words, the nerve seems to be able to develop only a limited amount of negativity, and if the negativity has been already driven up by previous stimulation (in the form of retention), the consequence will be a corresponding reduction in the size of the next action current, so that the same "ceiling" is always reached   Fig 2 illustrates this point   As the diagram shows, during the first short (3 seconds) interruption of the stimulus there is little decrease in the amount of retention present, and, correspondingly, there is little change in the size of the next action current   After longer rest (of more than

a minute) has been allowed, the retention subsides considerably, and, correspondingly, the next action current increases so as approximately to make up for the difference
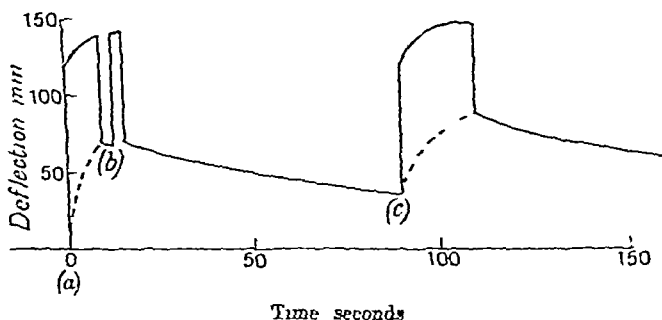
Fig 2. *Same nerve and conditions as Fig* 1 ' Ceiling" phenomenon, represented by the fact, that the maximum height attained by the action current is the same (a) after long rest (b) after 3 seconds rest only and (c) after 73 seconds rest In each case the reduction in size of the action current is compensated by the remainder of negativity (retention)

As has been stated, this phenomenon is not absolutely constant in its appearance The stimuli must be neither too short nor too long The effect of stimuli following each other so frequently that the retention has no time to wear off completely between the individual impulses is to produce a "staircase ' phenomenon, and this "staircase" will reach the "ceiling" only if a sufficient number of stimuli at a sufficient frequency be applied In the case of a tetanic stimulus only a few seconds are required to reach the "ceiling" On the other hand, a prolonged tetanic stimulus, as we know already, results in the total electric response (action current plus retention) starting to decline despite the presence of the stimulus, in which case the rule of the "ceiling' does not apply either (The reason for this decline will become apparent in connection with the "local fatigue ' described below) The phenomenon is also apt to be obscured by spontaneous changes in the injury current With the nerve, however, in good condition, and the tetanic stimulus neither too short nor too long, the phenomenon is very striking and can hardly be due to accident

The whole complex of these phenomena is highly suggestive of the accumulation of some products in the nerve during activity, their presence being responsible for the negative potential of the retention of action current and their elimination for the disappearance of the negativity The total amount of the products which may develop (possibly

from some other reserve substance) being limited, the amount of negativity is also limited   (Alternatively, the using up and slow restoration of some substance causing positive potential might be assumed to have the same effect)

(c) *Local fatigue and fatigue due to conduction*   In order to investigate how far the observed decrease in size of the consecutive action currents could be ascribed to fatigue of the whole nerve trunk as a result of the conduction of nervous impulses, and how far one had to deal with a purely local effect in the proximity of the stimulating electrodes, a chamber with two pairs of stimulating electrodes was constructed, on the lines of the one used by Garten and already referred to   With the help of this arrangement the experiments illustrated in Figs 3 and 4 were made
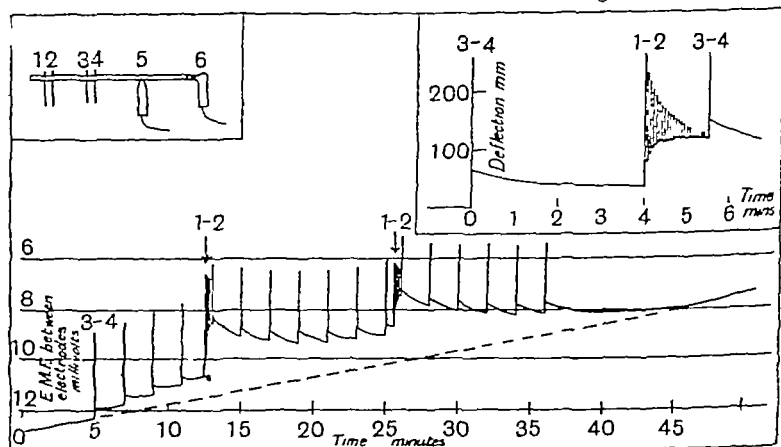
Figs. 3 and 4   *Experiments on "local" and "conduction" fatigue*
For explanation see text

Inset is the scheme of the electrode arrangement, 1–2 being the fatiguing pair, 3–4 the pair for short test stimuli only, and 5–6 the non-polarisable galvanometer circuit pair   In Fig 3 a test stimulus with electrodes 3–4 shows the presence of considerable excitability, then, at the fourth minute, a fatiguing series of rhythmically made and broken tetanic stimuli (2 seconds on and 2 seconds off) is administered from electrodes 1–2 until the almost complete disappearance of response Another test stimulus from electrodes 3–4 reveals the existence of considerable excitability at the corresponding part of the nerve and thus shows that the fatigue evidenced by the stimuli at electrodes 1–2 is largely

local At the same time, the size of the second test deflection is considerably smaller than the first one The distance between the electrodes 2 and 3 being 2 cm , this effect can hardly be ascribed to a spread of the injurious effect of the stimulus, the experiment thus shows that there is a certain degree of fatigue due to conduction of nervous impulses as well This has been definitely established by the experiment of Fig 4 in which care was taken to make sure that the nerve did not deteriorate of itself between the two test stimuli. To that effect a regular series of test stimuli of 1 second duration each was applied every 2 minutes The first four deflections are all of substantially the same size, thus proving that the nerve is in a steady state Then at the end of the next interval a fatiguing stimulus of $\frac{1}{2}$ minute duration is interposed from the electrodes 1–2 The response to the next test stimulus from the regular series is considerably smaller, thus showing the existence of fatigue due to conduction of impulses That recovery also occurs is shown by the fact that the next deflections of the series again increase in size The experiment was repeated later with the same result This experiment is also instructive in showing the gradual accumulation of retention in consequence of stimulation, and its gradual wearing off. The speed of this wearing off is seen to be greater the greater the amount of retention present, and follows an approximately exponential course The injury current during this experiment steadily declined from about 13 to 8 millivolts, as shown by the ordinates, which are in terms of injury current

One inference from these observations on local and conduction fatigue appears of general interest, viz , that the existence of local fatigue makes it difficult perhaps impossible to achieve a marked degree of fatigue along the whole of the nerve As soon as the nerve is exhausted locally, it ceases to send out impulses, and the rest of the nerve starts to recover instead of becoming further fatigued This evidently accounts for the fact mentioned when analysing Fig 1 that the retention does not increase indefinitely with the stimulus, but tends to reach a maximum, and then declines despite the continuance of the stimulus It appears also that the fact of the nerve showing a marked degree of local fatigue may, after all (provided care be taken to avoid injurious stimuli), be regarded as an indication of its general fatigability It is difficult or impossible to administer an artificial stimulus which will act as gently as a natural, conducted disturbance and a nerve is always bound to fatigue first at the spot where the stimulus originated , thus, owing to lack of excitation, it will be found less fatigued at all other spots

(*d*) *Distribution of fatigue and retention correlated*   The facts described
under (*b*) are by themselves highly suggestive of an intimate connection
between fatigue and retention of action current   It is hardly conceivable
that the parallelism in the degree of fatigue and in the amount of retention
present, as also the fact that the nerve has recovered when the retention
is reduced to zero, are only due to an accident   Some further evidence
also is available   On the one hand, experiments suggest that the amount
of retention of action current is a measure of fatigue, since the retention
and the reduction in size of the response vary in parallel, on the other
hand, it has been proved that the fatigue is not distributed uniformly
along the nerve but is greater in the proximity of the stimulating
electrodes   If the cause of both phenomena is the same, then the retention
also ought not to be distributed uniformly along the nerve but should
be greater in the proximity of the stimulating electrodes, in other words,
after stimulation, a point on the nerve near the stimulating electrodes
should be more negative than a distant point   In order to test the truth
of this assumption a chamber was constructed supplied with the usual
four electrodes but with the stimulating electrodes also non-polarisable,
provision being made for the possibility of switching different electrodes
into the galvanometer circuit   If we number the electrodes consecutively,
electrodes 1–2 were permanently connected with the stimulating coil,
and for the galvanometer circuit the combinations 3–4, 2–3, or 2–4 could
be promptly established   With this arrangement a great number of
experiments were performed under varying conditions   For instance,
electrode 3 was brought very close to 2 and the galvanometer circuit 3–4
established with the point 4 also uninjured   In this case, whilst a fresh
nerve shows practically no P D , electrode 3 becomes more negative
than 4 after stimulation, the usual picture of slowly receding retention
being observed on the scale   The same fact is revealed by using the usual
monophasic injured-uninjured lead-off and the customary relative dis
tance between electrodes 1, 2, 3 and 4   In this case the experiment is
conducted in the following manner   The injury current between 3 and 4
is first compensated until the galvanometer shows no deflection, then
its reading in connection with 2–4 is noted (it usually differs slightly from
zero), then, after the nerve has been stimulated, the readings of the
galvanometer in position 3–4 are compared with those in position 2–4
by quickly switching over from one position to the other   It is invariably
found that the increase of negativity (retention) of 3 with regard to 4
is smaller than the corresponding increase of 2 with regard to 4, and this
difference slowly wears off until, if the stimulus was not excessive, the

original state is reached   Another set of experiments was carried out by means of shifting electrode 3 along the nerve nearer to and further from electrode 2, and comparing the P D in different positions

These experiments have definitely established the fact of a greater negativity in the neighbourhood of the stimulating electrodes as the result of a stimulus

They have also accidentally revealed the fact that in the crab's nerve one always gets a partially "monophasic" effect even from a diphasic lead The direction of this effect appears to be, in the majority of cases, the same as the direction of the true monophasic effect in case of injured-uninjured lead   It does not apparently depend on the direction of the small current which is normally present between two uninjured portions of the nerve This is shown by an experiment, in which the electrode 4 was shifted nearer and further from the cut end, whereby a residual current of the same, and of the opposite, direction as the injury current was consecutively obtained, the direction of the "monophasic ' effect remained unaffected thereby This effect can also hardly be associated with the presence of cut branches, as the nerve was so dissected that no substantial number of fibres was cut Moreover, some experiments were performed in which the nerve was lifted from the electrodes and put back with its direction reversed, the direction of the "monophasic" effect was not affected thereby The direction of the "retention" always coincides with the direction of the "monophasic" effect, even in those few cases when the direction of the latter was reversed This seems to be another proof of the intimate connection existing between the action current and "retention," as it implies that at the electrode where the action current happens to be preponderant the "retention ' is preponderant as well   No reversal of the "retention" (equivalent to the positive after-swing) was observed so far The number, however, of experiments carried out with the purpose of clearing up the, no doubt, complicated problem of the "monophasic" effect is not yet sufficient, and an improved apparatus for recording the action currents seems indispensable

DISCUSSION

The retention of action current has been observed by many authors in various excitable tissues Du Bois-Reymond described a permanent diminution of the injury current in the frog's muscle (negative after-effect), and this was confirmed by Hermann(7)

As a result of my observations I made some preliminary experiments of frog s muscles and found it easy to obtain curves of retention of action

current very similar to those of a crab nerve, even retention after single shocks could be easily observed At the time I had no knowledge of being anticipated, in part at least, by the founder of electro-physiology himself

Samojloff(8) made experiments on frogs' hearts, in which periods of activity were interrupted by periods of rest of several seconds In some experiments the heart contracted normally, and periods of rest were brought about by stimulation of the vagus, in others the first Stannius ligature was applied, the heart being maintained in rhythmic contraction by induction shocks and periods of rest obtained by interrupting the induction shocks The heart was led off monophasically to a string galvanometer In both groups of experiments he obtained the movement of the base-line of the galvanometer during activity in the sense of the action currents themselves (decrease of injury current), and during the periods of rest in the opposite direction (increase of injury current) He thus confirmed the noted experiment of Gaskell(9), who was the first to discover that stimulation of the vagus in a tortoise heart brings about an increase of injury current, but while Gaskell interpreted this effect as being brought about by the increase of the anabolic processes of the heart under the influence of the vagus, Samojloff believes that rest alone is responsible for the gradual decrease of this state of negativity (which he identifies with a state of general excitation) brought about by the previous period of activity

As for nerves there is also no lack of observations to the same effect Biedermann (*l c*) says of the *Anodonta* nerve "At the close of the rhythmical excitation the magnet usually returns with decreasing rapidity to its position of rest, or there may in stale preparations be a negative remainder of the deflection" Garten (*l c*), in his monograph, gives many excellent capillary electrometer records of the olfactory nerve of the pike His analysed records show clearly in each case of a monophasic response a remainder of negativity, which takes much longer time to wear off than the action current proper The falling phase itself appears to consist of two distinctly different parts, whereas the initial part of it is of approximately the same order of steepness as the rising phase, the remainder of the falling phase appears practically horizontal at the speed of the recording surface used The same may be inferred from a mere inspection of many of his non-analysed records, taken at a slower speed There can be no doubt that the total electric response (action current in the sense of Garten) is composed of two separate parts the action current proper and its retention (in the sense of the

present investigation) This second part is affected independently of the first by various factors For instance, in Fig 48, showing the changes in the action current during the carbon dioxide narcosis, the steepness of the ascending phase of the action current (and also of the initial part of the descending phase before the retention sets in) is seen to remain unaltered through the narcosis (Whatever small difference there may be cannot be detected at the slow speed of the drum used) At the same time the wearing off of the retention is affected considerably, and from being very steep at the beginning of narcosis it gradually becomes horizontal at the height of narcosis, and then gradually returns to the previous steepness as the gas diffuses out Activity has a somewhat similar effect (the similarity of the effects of carbon dioxide and activity has been particularly stressed by Waller[10], and the drawn-out effect of activity on the descending phase has been observed by Boruttau[11]) So far, however, as I am aware Garten himself does not call attention to this complex nature of the electric response and to the possibility of the last part of it being connected with the processes of restitution In this respect his attention, and that of many other physiologists, was directed to the positive after-swing ("positive Nachschwankung ') discovered by Hering in 1864 and interpreted by him as being due to an increase in assimilatory processes in the nerve after stimulation.

In the frog s nerve the positive after-swing is very easily obtained, and, since Hering s discovery it has been an object of a long series of papers which resulted in no clearness or unanimity Woronzow[12] points out that the positive after-swing is a phenomenon of uncertain occurrence and the conditions of its appearance are difficult to fix. In every case its presence is associated with the negative after-effect due to the frog s nerve acquiring, during tetanisation, a certain negativity of a kind more stable than the action currents, this negativity taking 1 to 2 seconds to wear off after the stimulus is ended The positive after-swing he explains as a transitory increase of the negativity of the injured end after stimulation which superimposes itself on the receding curve of the negative after-effect thereby causing the curve to overshoot The negative condition of the nerve itself, on the other hand he interprets as a kind of persistent state of activity corresponding to the exaltation phase of Wedensky and Beritoff.

It appears, thus, that the primary phenomenon. connected with dissimilation in the sense of Hering is this accumulation of negativity, its slow disappearance is connected with restitution (assimilation), the fact that the electric potential, whilst changing, sometimes "over-

shoots the mark" is probably due to some secondary phenomenon, which will be better investigated, when the main facts are better known

The crab's nerve seems to lend itself very well to this kind of study, as in it the dual nature of the action current is particularly well represented If in quickly reacting nerves like the frog's sciatic, some doubts of interpretation may on occasion arise, owing to the partial fusion of the two phenomena, this can hardly be the case for the crab's nerve

An interesting confirmation, by a different method, of the existence of a second more persistent process in nerve was furnished by Verzär[13] by experiments on polarisation of nerve by constant current Bernstein discovered first that the extrapolar electrotonic currents decrease in strength when the nerve is stimulated On the other hand, Gruhnhagen observed that the intrapolar polarising current is increased thereby Both the Bernstein and the Gruhnhagen phenomena were explained by Hermann in terms of his law of polarisatory increment (or decrement) of the electric response in the nerve in the state of electrotonus Verzär repeated the experiments of his predecessors on extrapolar currents with modern methods and found that the decrease in their strength lasts considerably longer than the stimulus He suggested the decrease in polarisability of the nerve as the explanation of the pheno menon, the longer duration of it as compared with the action currents being due to a second process in nerve (The possibility of a decrease of polarisability occurred, however, already to Hermann) Samojloff[14] succeeded in investigating also the changes in polarisability (or resistance) of the intrapolar stretch of the nerve and confirmed the findings of Verzär for the extrapolar currents It seems that, in so far as this persistent effect is concerned, we are dealing with a phenomenon having the same origin with the "retention" considered in this paper A very comprehensive study of the polarisability of nerve has been made, with the help of the Braun tube oscillograph, by G H Bishop and J Erlanger[15] We will not attempt to summarise the contents of their paper, but it is of special interest here that they suggest that the rising phase is caused by the process of excitation, and the falling phase by the process of restoration, and both processes may be affected differentially by different factors The resting condition itself is a state of equilibrium between them. The present investigation on crab's nerve, and also other experiments quoted above, however, suggest that the falling phase itself is not uniform in its origin. The "retention"

of the action current, which in crab's nerve is so slow as not to allow any doubts of interpretation as a separate process, may escape detection in other quickly reacting nerves owing to fusion with the initial part of the falling phase

This short survey demonstrates—and no doubt instances could be multiplied—that the retention of action current is a phenomenon widely encountered in all kinds of excitable tissues The analogy between muscles and nerves is especially worth noting

Since the fundamental discoveries by Fletcher and Hopkins and by Hill and Meyerhof of the prolonged states of oxidative recovery in which the lactic acid produced in muscles is slowly destroyed, it is natural to imagine that the increased negativity of the tissue after activity is only another expression of the accumulation of the lactic acid It is hardly conceivable that changes of acidity in muscles, as large as we know actually to occur, would have no counterpart whatever in the changes of electric potential The important work of R Beutner(17) in which he succeeded in artificially imitating the biological currents by concentration chains, including water-immiscible phases ("oils"), proves that organic acids or their salts can create potentials of the right order of size by reason of their much greater capacity of penetration into the "oily" phase, than the capacity of inorganic acids or salts The origin of the potential he transfers, basing his views on Cremer's nitro-benzol chain, into the difference of composition of the membrane (or "oily" phase) itself, and not into the difference of the watery phases, as was done before, the actual seat of the potential being at the interfaces between "oily" and watery phases The addition of acid to the "oily" phase makes it positive with respect to the watery phase A double membrane, the outer one containing much acid, and the inner one containing none or little of it, accounts, according to his theory, for the origin of the injury current As to the specific nature of this acid, he makes no suggestions, lecithin, or oleic, or palmic acids, dissolved in guaiacol, were successful in imitating demarcation potentials in plant tissues in his previous work in collaboration with Loeb R Mond(18), however, succeeded in constructing chains, including tanned gelatine and other albuminous substances, which, in his opinion, reproduce the biological potentials better According to him and to some other research (quoted in his paper) done in the institute of Prof Höber, the large effect of organic ions on potentials, existing in the case of "oils," has not been confirmed on the biological objects, and the albuminous chains are more satisfactory in this respect It is, however, clear that much work

has to be done before any definite conclusions in this respect are arrived at For our subject the work of Beutner is interesting in supplying some experimental basis for the lactic acid theory of the origin of the "retention" of the action current, tentatively proposed here A develop ment of acid at the inner side of the Beutner's double membrane would explain the direction of the potential observed Beutner also found a considerable increase in the conductivity of the "oils" after their being impregnated by organic acids or salts This is in good agreement with the evidence existing as to decreased polarisability of tissues after stimulation, and may serve to further strengthen the "acid" theory of the origin of the "retention" of the action current

SUMMARY

(1) The excised limb-nerves of crustaceans (*Maia squinado* and *Cancer*) which are non-medullated, survive well and form a suitable object for electrophysiological investigation They are easily excited by induction shocks

(2) They are readily fatigued by tetanic stimulation, as shown by the nearly total disappearance of the electric response, and they recover if left at rest for sufficient time A fresh nerve is fatigued in about 2 minutes of tetanic stimulation, after the nerve has been exhausted by previous stimulation a few seconds suffice, 4 to 10 minutes are neces sary for the recovery of the nerve after sustained tetanic stimulation

(3) This fatigue goes hand in hand with an increase in the negativity of the nerve, additional to the negativity of the action currents and of a more persistent nature Each single impulse, by itself of very short duration, leaves behind it a state of negativity ("retention of action current"), wearing off in a few seconds, this negativity accumulates if the stimuli follow each other frequently enough ("staircase" pheno menon), and it then takes a longer time to wear off

(4) The greater the amount of the "retention" present the greater is the reduction in size of the electric response ("ceiling" phenomenon) The recovery goes hand in hand with the wearing off of the "retention," and the disappearance of the "retention" is a sign of complete recovery

(5) The experimental fatigue of crustacean nerves is a combination of local and conduction fatigue It is considerably greater in the proximity of the stimulating electrodes, but the whole nerve is also fatigued as the result of conducted impulses

(6) It is suggested that the phenomenon of local fatigue is an indica tion of a nerve's general fatigability

(7) There is a complete parallelism in the distribution of fatigue along the crab's nerve and in the distribution of "retention" of action current The "retention" (negativity) is also greater in the proximity of the stimulating electrodes

(8) A survey of literature demonstrates that the retention of action current is a phenomenon widely encountered in all kinds of excitable tissues It is probably connected with processes of restitution

It is my pleasant duty to express my indebtedness to Prof. A V Hill for his kind interest and help throughout this investigation My thanks are due also to Dr E J Allen and to the staff of the Marine Biological Station in Plymouth for the courtesy extended to me during my stay in Plymouth

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THE EFFECT OF FREQUENCY OF STIMULATION ON THE HEAT PRODUCTION OF NERVE

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In the experiments previously reported by Downing, Gerard and Hill[1] on the heat production of nerve the stimulating agent employed was a Harvard coil in which the ordinary spring had been replaced by one which gave 140 complete vibrations per second, so supplying 280 shocks per second, 140 make and 140 break It was assumed that, up to the limit where a stimulus would fall within the absolute refractory phase of its predecessor, the heat production would increase with frequency of stimulation, and, since the measurement of the heat is by no means easy, it was desired to have as much heat as possible to measure In occasional experiments the ordinary Harvard coil was employed, giving 100 stimuli per second, 50 make and 50 break, and we noticed always that the heat production did not fall off nearly as fast as the frequency between 100 and 280 stimuli per second the total heat fell off only in the ratio of about 3 to 2 Since in dealing with the mechanism of nerve activity it is desirable to know the heat-production per impulse, it is necessary to divide the observed total heat by the frequency in order to obtain what one requires, and it became obvious that this would not be constant as the frequency changed, so that the heat per impulse required further definition Nearly all work on the nervous impulse (apart from that of Erlanger and Gasser and their colleagues[2, 3]) has been concerned either with single impulses, or with pairs of impulses, so that from this point of view it was obviously desirable to find the heat per impulse when unaffected by the presence of other impulses, and to study the rate at which the energy liberation in the impulse "recovered" after a previous stimulation The heat production, however, in a stimulated nerve is so small that there is no hope at present of being able to determine it directly in a single impulse It is necessary

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to work with a long succession of impulses, several hundred at least, in order to obtain any accuracy, and these must follow one another in fairly rapid succession, since the rate of heat loss of the thermopile is so great, thus it is essential to know the relation between frequency of stimulation and heat production in order to proceed further Incidentally, the investigation of the relation has supplied some interesting information about the "recovery process" which is completed in nerve during a fraction of a second after stimulation[1]

Method It was necessary to obtain some suitable means of applying stimuli to a nerve at a rate varying from (say) 20 per second up to (say) 500 We attempted at first to use a valve-generator, supplied by the Cambridge Instrument Company, which produces an alternating current approximating to a pure sine wave, the frequency of this can be varied as desired, by changing the capacity of a condenser The higher frequencies were easily attainable with this arrangement, but the lower frequencies provided a difficulty Moreover, the stimulating effect on nerve of a pure sine wave current is very poor when compared with the energy it contains For many purposes there is no harm in using such a means of stimulation, because no polarisation results from an alternating current, and even though it contains an unnecessary amount of energy it does not injure the nerve, while in most types of experiments the heat liberated by the stimulating current is of no importance In the present experiments, however, it was essential that the stimulating agent should liberate the minimum of energy in the nerve, because if the energy so liberated is excessive it conducts down from the stimulating electrodes, reaches the thermopile in spite of all precautions, and causes serious errors in the results A more satisfactory agent is a series of extremely rapid induction shocks, as supplied by a coreless coil. With such a coil the stimulus is produced very suddenly by the rapid rise of the induction shock, and no energy is wasted in the interval between successive stimuli, as is the case when an alternating current is passing continuously between the stimulating electrodes

The ordinary vibrating spring, with a pointer moving in and out of a bowl of mercury, we did not regard as a sufficiently accurate and reliable means of regulating the shocks, since it is difficult to ensure either that make and break shocks are equally spaced, or that they are

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[1] The use of the word "recovery" in this sense is well established, as also is the term recovery" in muscle in a completely different sense The "initial recovery" of nerve, complete in a few hundredths of a second, has no relation to the ' delayed recovery process' which occupies ten minutes or more.

equal in magnitude, and there is no easy way of eliminating the make shocks if desired   We arranged therefore for the construction of the contact-breaking device shown in Fig 1, which has proved extremely
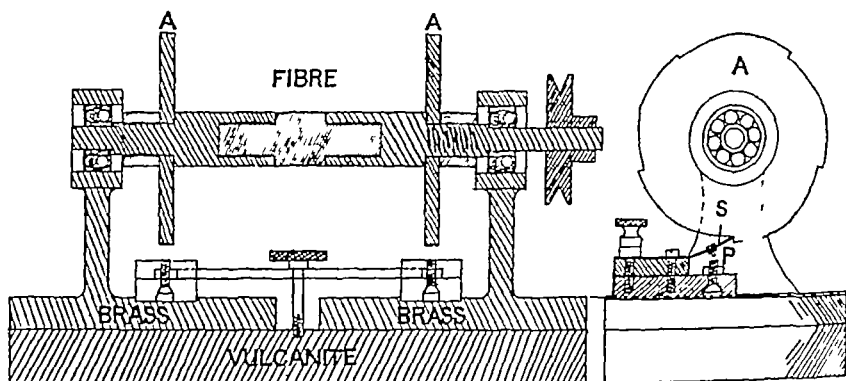
Fig 1. Revolving contact-making and breaking device, carrying two steel discs *A A* insulated from one another on the same shaft, each supplied with five cams for making and breaking contact at the point *P* carried by the phosphor bronze spring *S*

satisfactory   As designed, there are two circular discs running, insulated from one another, on the same shaft, each containing five cams which lower the tip of a phosphor bronze spring and then suddenly release it, so making and breaking contact at regular intervals[1]   It was expected that it would be necessary to employ only break shocks in the investigation, so that one of the cam discs was designed to make and break the primary circuit of the coil, as it revolved, while the other cam disc was intended to short-circuit the make shocks (or, if desired, the break shocks) only   As a matter of fact, we found it unnecessary, except for a few special purposes, to employ both discs, and have used both make and break shocks, which, with the coreless coil employed, have proved to be practically equally effective, this has the advantage also of avoiding polarisation in the nerve

The cams and springs were so arranged that the contact, which was between two platinum points, occurred almost exactly half-way between two breaks   This could be tested by eye, or better by putting a voltmeter across the contact and marking the points at which a deflection occurred when the wheel was slowly revolved by hand   The breaks were very sharp, being caused by the spring falling off the end of the cam

[1] For the success of the instrument we are mainly indebted to the skill of Mr A. C. Downing

It was obviously necessary to ensure that the shocks were approximately equal at all frequencies For this purpose a very fine resistance wire, wound non-inductively round the junctions of a thermopile, was employed, the current from the induction coil ran through the resistance wire, so warming it and thereby the junctions of the thermopile, the E M F from which gave on a moving-coil galvanometer a deflection proportional to the energy in the induction shocks Setting in motion the contact-making and breaking device, connected in the ordinary way with a coil, and reading its speed (and so the frequency of the shocks) with an accurate tachometer, the energy per second in the induction currents from the secondary could be compared with the frequency If the shocks were equal at all frequencies the total energy read on the galvanometer scale would be proportional to the frequency, and this was found actually to be the case up to frequencies of 400 shocks (200 make and 200 break) per second Beyond that limit the total energy continued indeed to increase with the frequency, but at a diminishing rate, the energy per shock becoming somewhat less The coil employed was coreless, so ensuring quickness of rise and fall in the primary current and rapidity in the induction shocks

A similar method was employed in order to be sure that the make and break shocks were equally effective For this purpose the energy of the make shocks alone was determined, by short-circuiting the break shocks by the second cam-disc and contact, or the energy of the break shocks alone was determined, short-circuiting the make shocks These came out about the same and equal to about half the energy found when both make and break shocks were employed Since the total amount of current passing in a make shock is necessarily the same as that in a break shock (for a given resistance, coil distance, and primary current), the equality of the energy in the two shocks shows that they were practically of the same form, and therefore would have the same stimulating efficacy

The stimulus was varied by altering a resistance in the primary circuit, the secondary being fixed over the primary Supermaximal shocks were employed, as large as it was practicable to use without producing "current heat" in amount sufficient to conduct from the stimulating electrodes down to the thermopile, and so cause an error in the result It is necessary, at high frequencies, to use stimuli which are very considerably supermaximal, because the excitability of the nerve takes an appreciable time after a stimulus to attain its original value In all previous experiments with the Harvard coil giving 280

shocks per second, the position for maximal stimulation at that fre
quency was known Employing our contact-making device at the same
frequency, we were able to show that it produced the same response as
the coil for considerably weaker currents than we actually used in the
following investigation For frequencies, therefore, of 280 per second
and below, our stimuli were certainly maximal, and probably also for
a considerable range above that frequency

The contact-making and breaking device was driven by a motor
through a pair of countershafts carrying cone pulleys, so that any
desired frequency of revolution could be obtained The constancy of
speed as read by the tachometer was amply sufficient for our purpose
All speeds were read as revolutions per minute of the cam arrangement,
and multiplied by 10 (5 make and 5 break shocks per revolution) and
divided by 60, that is altogether divided by 6, to obtain the number
of shocks per second

With the extreme sensitivity required for these experiments on the
heat production of nerve, difficulty is often experienced from electric
leaks, and it was found necessary to remove the whole of the stimulating
arrangements to the next room and to bring only a flexible wire, carrying
the make and break induction shocks, into the experimental room Two
observers therefore were needed, one to read the scale and to manipulate
the apparatus for measuring the heat, the other in the next room for
adjusting and measuring the speed and giving 10-second stimuli The
period of stimulation was always the same, the observer, employing a
stopwatch, pressed a Morse key in the primary circuit and released it
at the end of 10 seconds, giving appropriate warning to the observer
at the scale in the next room

The accuracy with which the heat can be read, especially at low
frequencies where it is small, is relatively low, so that it is necessary
to make a large number of observations The relative infatigability of
nerve enables large numbers of observations to be made, the most
reliable condition being that stimuli should be spaced at regular time
intervals, for example of one or two minutes, in that case hundreds of
observations can be made on a single set of nerves, passing several times
up and down the range of frequencies desired It is not practicable to
work at frequencies of less than about 20 stimuli per second, because
below that limit the heat becomes so small that random errors due to
extraneous disturbances render the results unreliable, and we have not
considered frequencies above 400 to 500 per second, for at such fre-
quencies we pass into the range where many of the stimuli must be

ineffective, for example, every alternate one falling in the completely refractory phase left by its predecessor So far as our experiments go, the heat production still continues to increase slightly, however far the frequency is pushed. Owing, however, to the theoretical complication introduced by the nerve no longer following the stimuli at the higher frequencies, we limited our observations to the range, 20–420 per second It is obvious, of course, that the origin of zero frequency must lie upon the curve

Results The individual observations are so numerous that they cannot well be recorded here. Their number, however, ensures that random errors due to extraneous causes do not appreciably affect the results, and we have taken a mean for the following frequencies from the smoothed curves of several experiments In each case the curve was reduced to the same scale before reading off the value for calculating the mean All experiments were made at about 18° C

TABLE I. Mean values of heat (arbitrary units) at different frequencies (stimuli per second), from which Fig. 2 is constructed.

Frequency	0	20	40	60	80	100	140	180	220	280	360	420
Heat	0	23	40	52	61	67	77	82	87	93	97	99
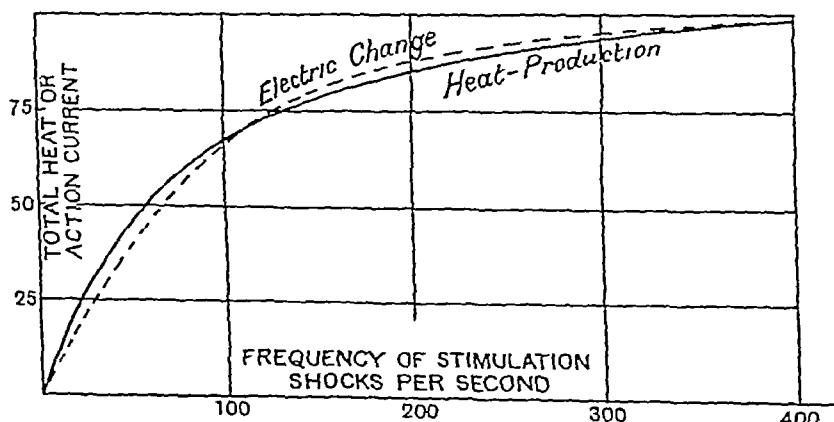
Fig 2. Relation between total heat per second (full curve), or total action current per second (broken curve) and frequency of stimulation in shocks per second. The scale of each curve is arbitrary, but the two curves are made to coincide at a frequency of 400

The result is shown in Fig 2, where there is also given for comparison a curve of "total" action current obtained in the manner described below

The curve in Fig 2 is in arbitrary units of heat We can interpret

it in absolute units, for maximal stimulation of a frog's nerve, by using the data of the paper by Downing, Gerard and Hill[1] There it was shown that one second of maximal stimulation at about 15° C , at a frequency of 280 per second, liberates, of initial heat, about $7\,6 \times 10^{-6}$ calorie per grm., of total heat, that is initial plus delayed, about 69×10^{-6} calorie per grm   Assuming these values at 280 stimuli per second, we may calculate those for any other frequency The result is given in the following table expressed in terms of heat per impulse, the heat per second being obviously obtainable by multiplication by the frequency For the purpose of the argument which follows we give also, in the second column, the interval between stimuli. The last entry, that at zero frequency, refers to the case of a single isolated impulse unaffected by any previous stimulation of the nerve It is obtained by laying off the tangent at the origin to the curve of Fig 2

TABLE II. Absolute values of the heat per impulse at different frequencies, reckoned per gram of nerve.

Frequency (shocks per sec)	Interval between shocks (σ)	Initial heat $\times 10^{-6}$ cal.	Total heat $\times 10^{-6}$ cal.
400	2 5	2 03	18·4
350	2 86	2 27	20 6
300	3 33	2 56	23 2
280	3 57	2 71	24 6
250	4 00	2 98	27 0
200	5 00	3 47	31 5
150	6 67	4 30	39 0
100	10 0	5 53	50 2
80	12 5	6 28	57 0
60	16·7	7 19	65 2
40	25	8 26	75 0
20	50	9 49	86 1
0	∞	10 32	93 7

The entries in the last two columns are smoothed values, and so are given to three significant figures.

The most interesting entry in Table II is the last one, showing that in a single isolated impulse in a nerve there is a rise of temperature, corresponding to the initial heat, of almost exactly one ten-millionth of a degree Centigrade, while the total heat (initial plus delayed) in a single impulse is nearly one millionth of a calorie per grm These may be compared with the case of a single muscle twitch, where there is an *initial* rise of temperature of about 3/1000 of a degree, 30,000 times as much as for the nerve, and a *total* heat of about $7\,5 \times 10^{-3}$ calorie per grm , about 8000 times as much

Similar experiments have been performed, measuring the total action current instead of the heat The term "total action current" requires

some explanation. The nerve, mounted in a suitable chamber, and arranged to give a monophasic response, with two non-polarisable electrodes of the zinc-zinc-chloride gelatine-Ringer type, was stimulated at a point distant from these electrodes and, to use the classical term, the "negative variation of its injury current" was recorded on a sensitive high resistance galvanometer The instrument employed was a Downing galvanometer(3) of very high resistance (20,000 ohms), the extreme sensitivity of which allowed a deflection of 30 mm. or more to a single shock, in spite of the rapid movements (half a second deflection time) The injury current of the nerve was balanced and the galvanometer brought to zero, a stimulating current from the make and break arrangement described above being allowed, by means of a Lucas revolving contact-breaker, to pass for half a second through the nerve The deflection obtained was read ballistically on the scale The readings represent the total area of the action-current-time-curve, above a base line corresponding to the balanced injury current In other words, the "total action current," as we have called it, measures $\int Cdt$ where C is action current, and t is time and the limits of the integral are the beginning and end of activity in the nerve

The process described above in reference to the measurement of heat was repeated in respect of electric change on a number of nerves Twelve reliable experiments were performed on twelve different nerves, and each of the curves relating electric change to frequency of stimulation consisted of a sequence of observations with increasing frequency, followed by another sequence with decreasing frequency, so as to eliminate as far as possible any effect of fatigue, or of change in the nerve These curves were then averaged and the resulting mean curve is shown by the broken line of Fig 2 The scale adopted is arbitrary, the curve of electric change being made to coincide with that of heat production at a frequency of 400 per second In nearly all respects, except that a different chamber was used, these experiments on the electric change are comparable with those on the heat, the stimulating arrangement and the temperature being the same, *Rana esculenta* being employed in either case The only difference, and it is one which can scarcely affect the results, is that the curve of electric change corresponds to stimuli of only half a second duration, while that of heat corresponds to stimuli of 10 seconds' duration No fatigue, however, occurs in a nerve in such a short interval of stimulation as 10 seconds, so we may regard the two curves of Fig 2 as being strictly comparable with one another

Discussion One noticeable characteristic of the heat curve in Fig 2

is the way it starts to bend round soon after leaving the origin. According to Lucas, Adrian, Kato and others, the relatively refractory period of the quickest fibres of a nerve, following an excitation, is over, or very nearly over, at 20σ At the corresponding frequency of 50 per second the heat per impulse as here measured is obviously falling off Clearly, as regards heat production, the "recovery process" (in the sense in which that term is employed by Lucas and Adrian) of all the fibres of a nerve considered in the aggregate, takes longer than does the "recovery process" of the quickest fibres in respect of excitability It is useful, therefore, to express our results in another way, in which the recovery of the capacity for liberating energy can be studied

If we take the data of Table II and plot the heat per impulse against the interval between successive impulses, regarding the heat at zero frequency as being 100 p c , we obtain the curve marked "return of energy liberation" of Fig 3   It is not possible to follow this curve
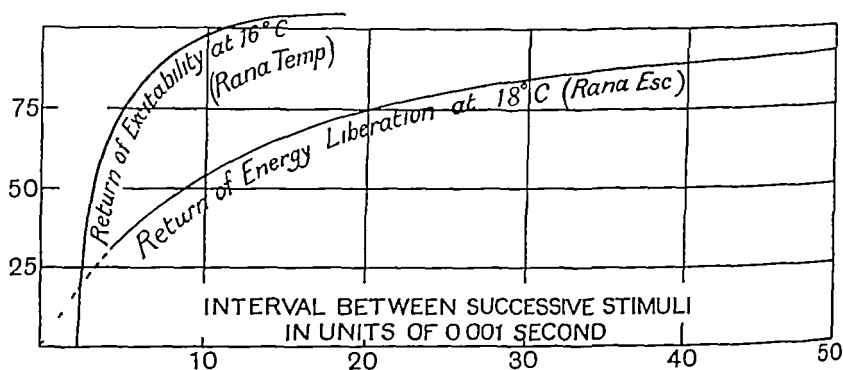
Fig 3   Return of energy liberation and return of excitability For the former the heat per impulse is given as a function of the interval between successive stimuli and is calculated from the curve of Fig 2 The return of excitability is derived from data supplied by Dr Adrian which were obtained from measurements of the least interval for muscular summation for different strengths of second shock.

experimentally to the right with any accuracy, for the reason given above, namely that at very low frequencies the total heat per sec is so small (however large it may be per impulse) that random errors affect the result The curve is dotted in below 4σ, as with intervals less than this some, at any rate, of the stimuli must be falling in the absolutely refractory phase of their predecessors Within the range, however, of 4σ to 50σ there is no doubt of the general form of the curve The initial energy liberation of the nerve, judging from the duration of the rising

phase of the action current (as shown by Bishop, Erlanger and Gasser(2)), is very short, say of the order of 0.75σ If we neglect it completely we may perhaps regard the origin as lying upon the curve, for at zero time after a previous stimulus the nerve has obviously not recovered at all its power of liberating energy If we regard the initial breakdown process as lasting for 0.75σ, a point distant 0.75 along the horizontal axis should lie on the curve Our present knowledge is not adequate to allow us to distinguish between such alternatives If, however the initial liberation of energy be complete within $x\sigma$ then the point $(x, 0)$ should lie upon our "recovery" curve For the present we have drawn it as a broken line passing through the origin.

The recovery curve of energy liberation does not obey exactly any simple formula and it is probably composite in its nature It represents presumably the average recovery of a number of fibres differing widely in their time relations According to Bishop, Erlanger and Gasser(2) some of the fibres in the frog's sciatic nerve take about four times as long to recover as do the most rapid fibres As a rough approximation the curve might seem to be exponential in character, corresponding to the assumption that the rate of recovery at any moment is proportional to the amount of recovery still to be completed The curve, however, rises initially more rapidly, and later more slowly, than it should on such an assumption though this would be explained if it were compounded of a series of exponential curves of different time-relations There would seem at present to be insufficient evidence to warrant any further discussion of its physico-chemical basis

The recovery of excitability of a nerve after a stimulus follows the relation described by Lucas and Adrian and investigated by many observers since The particular curve shown in Fig 3 has been supplied by Dr Adrian as a typical recovery-of-excitability curve for English *Rana temporaria* The nerves actually used in our investigation were those of Dutch *Rana esculenta* It is obvious that the return of excitability under their conditions follows a very different relation from the return of energy liberation under ours It should be noted, however, that the curve giving the return of excitability refers to those fibres of the nerve trunk which recover their excitability most rapidly—the observations on which it is based involve the measurement of the least interval required for minimal muscular summation, for given strength of second shock. The curve, on the other hand, of return of energy liberation refers to all the fibres of the nerve, since maximal stimuli were employed, at any rate for intervals greater than 4σ It may well be

the case that, taking the difference between the conditions into account, the two curves of recovery are fundamentally the same in other words, that if it were possible experimentally to observe the heat only of those fibres which recover most quickly, the curve of recovery of heat would nearly reach its asymptote also within 20σ or so

In Fig 2 it is seen that the total electric change follows very much the same course when the frequency alters as does the heat production What the significance may be of this relatively close agreement it is not possible as yet to say The similarity of the two curves of Fig 2 ensures that if we calculate a curve of return of electric change, similar to that of the return of energy liberation of Fig 3, we shall find the return of electric change to be similar in general to the return of energy liberation.

In view of the demonstration by G Kato and his colleagues of the complete applicability of the "all or none" principle, when expressed in the form that the size of the impulse in a nerve fibre is a function only of the condition of the fibre and is independent of the stimulus strength or of the distance the impulse has travelled, we may assume that all the impulses actually started in the nerve traverse the whole of the nerve and are not abolished by decrement Kato[4] in his second monograph, chapter VIII, deals with "the recovery of nerve," and proves, p 115, what is important for the present purpose, that in the case of three shocks given in succession the second least interval for muscular summation is equal to the first least interval, and therefore that "the refractory period due to the first disturbance of normal size evoked in normal resting nerve is equal to the refractory period due to the second disturbance of subnormal size evoked in the incompletely recovered nerve " If this conclusion can be generalised we are justified in following the process of recovery, as we have done above, by employing a rapid succession of stimuli, instead of a single pair Kato's result, moreover, suggests (p 116) "that the nerve, if it will respond at all to a stimulus, will discharge all that is available at the moment, independently of the strength of the stimulus and independently of whether it is in the resting condition or in the relatively refractory period "

Let us imagine that the total energy available in a nerve for immediate discharge is liberated suddenly on the application of a shock and that as soon as the "explosion" dies down the nerve begins to recover its power of liberating energy along the curve shown in Fig 3, or, in the case of a single fibre, along one of the elementary curves of which the curve of Fig 3 is compounded Let us imagine also that the impulse cannot propagate itself at all unless there is a certain amount of energy

available in it   A simple physical analogy is a train of gunpowder which will conduct a wave of burning, if ignited at any point, provided that the thickness of the train is sufficient   If the train be too narrow the combustion will die away at once and will not be transmitted   If this analogy be valid we obtain a simple picture of nerve activity, namely, that after an impulse a recovery process (presumably anaerobic) has to go on, restoring the nerve, as regards energy, to its initial condition and taking 50σ or more (or in the case of the individual fibres, 20 to 80σ) in the process, and that the absolutely refractory period is due to the fact that the wave will not propagate at all unless there be a certain amount of energy available

The energetics of the recovery of nerve to its initial condition following a stimulus (apart altogether from the delayed process which lasts for ten minutes or more after stimulation) provide an interesting problem   In the case of muscle we know that contraction is accompanied by a large and sudden liberation of heat, while relaxation, which occurs more slowly, is also accompanied by a liberation of heat which seems to follow the course of relaxation, at any rate approximately   In their paper on the nature of the isometric twitch Hartree and Hill (5, fig 6, p 406) describe the return of muscle, following a shock, to its initial state of capacity for liberating energy   The return of heat liberating power in a frog's muscle at $10°$ C is complete in about 0 2 sec, which is just about the time taken in complete relaxation at that temperature   It would seem likely that this "recovery" in muscle is associated in some direct way with relaxation, and, since we know that relaxation is accompanied by heat production, it may well be that in nerve the return to its initial condition will also be accompanied by a production of heat Thus, the process of "recovery" exhibited by the curves of Fig 3 may not be one involving no change of energy, indeed upon general grounds it would seem more likely that the chemical reactions involved in the restoration of nerve to its original condition would be accompanied by a liberation of heat, i e by a wastage of energy   Thus, in saying that in the initial process of a single impulse a nerve liberates about one ten-millionth of a calorie per grm, we do not imply that the whole of this energy liberation is confined to the interval during which the action current occurs, since there may be an initial rapid outburst of heat, as there is in muscle, followed by a slower liberation of heat occurring during the "recovery," which is analogous to relaxation, and takes 50σ or more (20 to 80σ) to complete   In this sense, therefore, there may well be, as in muscle, *three* phases of liberation of heat, namely, an initial and

very rapid phase concerned with the process which produces the rise of the action current, a second phase concerned with the restoration of the nerve to its original condition of excitability and conductivity and complete in 50σ or so, and a third phase lasting for 10 minutes, during which eight-ninths of the total energy is liberated   It is impossible at present by direct methods to analyse the heat as between these first two phases In the case of muscle, where the heat is much larger, and contraction and relaxation may be considerably displaced in time from one another by taking appropriate slow muscles and by lowering their temperature, the analysis is technically possible   In the case of nerve, where the heat in one impulse is very small and the whole process lasts only 50σ or so, there would seem to be no chance of an experimental isolation of these two first phases from one another   On the somewhat uncertain basis, however, of an analogy with muscle one may imagine the return of the capacity of the nerve for liberating energy to correspond to relaxation of the muscle, and so to be accompanied by a production of heat

Summary

1   The relation has been determined between the heat production of a nerve stimulated by a sequence of maximal induction shocks and the frequency of stimulation   The heat production per second continues to increase with the frequency but at a diminishing rate, the heat per impulse falling off as the interval between stimuli gets less   At a frequency of 280 shocks per second, as employed in the experiments of Downing, Gerard and Hill, the heat per impulse is not much more than one quarter of what it is at a very low frequency   From the relation observed it is possible to calculate the heat in a single isolated impulse In frog's nerve the initial heat for a single maximal impulse is about one ten-millionth of a calorie per gram, and the total heat about one millionth of a calorie per gram

2   From the results obtained it is possible to calculate a curve giving the return of the power of liberating energy, in a nerve impulse started by a maximal shock, as a function of the interval between it and its predecessor   This curve is of a composite nature, since some of the fibres of a frog's sciatic nerve take about four times as long to recover as the most rapid ones   Perhaps for this reason the curve is very different from that for the return of excitability as determined for the most rapid fibres by a second stimulus following a first one

3   The "total action current" (the negative variation of the injury current), as determined by an ordinary galvanometer for a given period

of stimulation, bears a relation to the frequency of stimulation which is very similar to that shown by the heat liberated

4 It is pointed out that the "initial recovery" of nerve following a stimulus is probably accompanied by a liberation of energy just as the process of relaxation in muscle is There are probably three phases in nerve activity, corresponding to contraction, relaxation and "delayed recovery" in muscle •

5 It is suggested that, after an impulse has traversed a nerve, an anaerobic recovery process (analogous to relaxation in muscle) has to go on, restoring the nerve to its initial condition as regards power of liberating energy, and that the absolutely refractory period is due to the fact that a wave will not propagate at all unless there be a certain amount of energy available.

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THE STRETCH REFLEX AS A SPINAL PROCESS

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THERE is an amount of evidence to show that in skeletal muscle there are end-organs whose adequate stimulus is stretch of the muscle [1, 2, 7, 9a, 10, 11, 12, 15a, 18] By their agency the muscle-fibres may be reflexly thrown into contraction [8, 10, 11], and may develop a continued active tension in response to continued mechanical stretch which, it has been argued, is the "tone" of the muscle [5, 8] This so-called "stretch-reflex" elicited by stretching the muscle tendon is, in fact, "tonus in its making" [9] The reflex was first investigated in animals decerebrated at the intercollicular level [10] As is well known, such animal preparations show a "decerebrate rigidity" of the antigravity muscles [16] which has been regarded as an exaggerated tonus The presence of the stretch-reflex, however, was also observed in preparations whose level of transaction of the brain stem was 4–5 mm. in front of the anterior colliculi In such preparations there is no hypertonus of muscle, but a normal distribution of tonus [4, 13, 14] In them the tension developed reflexly by the muscle in response to stretch is as well marked as in the rigid hypertonic muscle

It has long been known that in some animal preparations, especially "spinal" dogs, there is indubitable evidence of a tonic activity of anti-gravity muscles which depends solely on the integrity of nervous connection of those muscles with the isolated segments of spinal cord The "lengthening" and "shortening" reactions, reflex standing and walking, occur in these spinal preparations and are not far dissimilar from the activities displayed by a hypertonic decerebrate preparation [17, 18, 19, 21, 22, 23, 24] The knee-jerk, a reflex phasic response to a quick stretch or tap on the patellar tendon, is elicitable in intact animals, thalamic, decerebrate, and all spinal preparations But the long-lasting tonic response to continued stretch has not yet been recorded for spinal preparations, and it became of interest, therefore, to enquire whether the maintained stretch-reflex was elicitable in a spinal preparation after the subsidence of "spinal shock" [20] It has been

recorded that fleeting evidence of a positive character was obtained even a few days after spinal transection(9), though this was one positive observation among not unnumerous negative ones In the present communication are related the findings of experiments performed on "spinal" dogs two and three months after spinal transection, which showed a good degree of recovery from spinal shock such as the antigravity activity of their extensor muscles, reflex standing, reflex stepping, the extensor thrust(19), the lengthening and shortening reactions and Philippson's crossed reflex(15)

Method The same table with the descending top was used as previously(10,11,8) The myograph was not dissimilar, being a shadow myograph of high vibration frequency of 1000 D V per sec , with complete damping in 2 σ and magnifying the tendon-movement (on the plate) from 105 to 240 times according to the distance of the tendon pull from the axis of the myograph The usual rigidity of fixation of the preparation with drills was continued, and care taken to isolate or immobilise by nerve section all muscles in the neighbourhood of observation and, similarly, to denervate skin-fields, as far as possible The animal preparations (dogs ♀) had been made some 60 to 85 days previously by isolation of the lower region of the spinal cord with strict asepsis under profound general anæsthesia The level of section was either between 1st and 2nd or 2nd and 3rd post-thoracic roots The condition which was especially anticipated, viz "rigidity" of the knee extensor, was observed within 30 days of the section, being present as a well-defined "clasp-knife" rigidity Within another 30 days this rigidity at the knee became less marked until eventually it was quite slight, though plainly present The gastrocnemius muscle, on the other hand, showed most marked rigidity and an extreme resistance to the movement of flexion throughout the post-operative period The "lengthening reaction" therefore, though present, was difficult of elicitation in the vastocrureus muscle On these grounds, it was decided beforehand to observe at experiment primarily the effects of stretch on the gastrocnemius muscle, leaving the vastocrureus muscle as a secondary field of investigation The reason of the relative absence of rigidity in the vastocrureus muscle may have been due to impairment of its motor centre in the cord (4th and 5th post-thoracic) by the proximity of our section, but we could come to no definite conclusions on this point The gastrocnemius muscle, on the other hand, in the dog does not receive innervation from any level higher than the 6th post-thoracic segment In this connection, it may be recalled that even with sections of the cord in the cervical region

very marked rigidity of the ankle extensor may occur ((18), pp 817 and 825) A second possible reason for the relatively weak rigidity of the knee extensor may have been that during the post-operative period all the preparations maintained an extension of the ankle and an acute flexion of the knee as their ordinary resting posture This chronic flexion of the knee may well have impaired the stretch organs of the knee extensor

Results I Fig 1 shows the type of myogram obtained as a result of stretching by some 4 or 5 mm. a muscle whose total length from origin

Myogram of paralysed muscle
-          ,,   ,, muscle inhibited throughout application of stretch.
— — — — — Table fall indicator
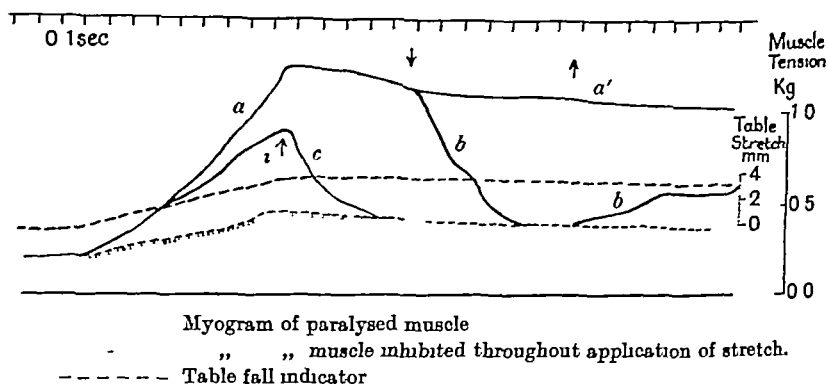
Fig 1 *aa'*, myogram of the gastrocnemius muscle Dog 2¼ months after spinal section 2nd–3rd post-thoracic segment, stretch of 4 mm slowly applied, as shown by table indicator *abb*, similar response to stretch inhibited by gentle pull on tendon of the tibialis anticus muscle (pull "on" ↓ and "off" ↑) *c*, similar, with tetanic inhibition applied throughout to ipsilateral posterior tibial nerve at ʀ↑ The smaller response to stretch before onset of inhibition is due to application of electrodes to and exposure of the nerve (see text)
The myograms of the muscle paralysed by nerve section and the muscle inhibited *ab initio* run closely parallel Tendon movement magnified 135 times.

to insertion is 12 to 14 cm. Its general features at once suggest that reflex activity of tension development which has been named the "stretch-reflex," "kinetic" or "phasic" during the application of the stretch and "static" or "tonic" during its maintenance The criterion of the reflex nature of the tension-development, is, as always, section of the muscle's motor nerve The myogram of the muscle so paralysed establishes the fact that the great bulk of the increase of tension in the non-denervated muscle is of reflex origin Further evidence for the reflex character of the process has been obtained by tetanic stimulation of an appropriate inhibitory nerve Reflex inhibition so produced annuls the muscle's activity down to a resting level of tension which corresponds

precisely with that of the muscle paralysed by nerve section. If the inhibition is produced before the mechanical stretch is given, the myogram traced is again in close correspondence with that of the denervated muscle Even a maximal single break shock applied during the plateau of a stretch reflex sufficed to inhibit a reflex tension of 1250 grm. completely for 0 3 sec , recovery taking place in a series of clonic steps reaching a tension of 900 grm. in 3 6 sec During the first 0 8 sec of this recovery, a clonic step occurred approximately every 0 2 sec , during the next 0 5 sec the rate was twice this and subsequently four times (Fig 2)
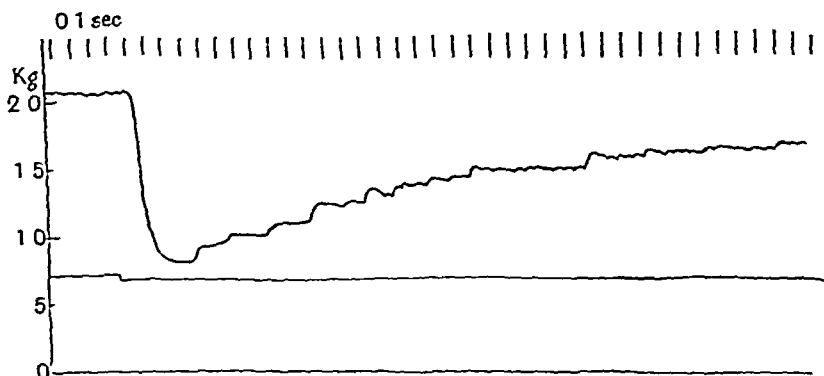
Fig 2. Inhibition of the stretch reflex by single break shock (10 cm. coreless Berne coil, primary 2 v) and subsequent recovery (see text for description) Tendon movement magnified 158 times. Of the total tension of 2100 grm., 850 grm. is passive tension, 1250 grm. active reflex tension.

An addition to our data regarding the reflex nature of the responses to stretch would have been the de-afferentation of the muscle under investigation by section of the posterior spinal nerve-roots That factor has not yet been pursued There seems to be, however, a sufficiency of evidence in favour of the definite existence of a stretch-reflex in "spinal ' preparations

II Although flexor muscles in various conditions of experiment respond to stretch of their tendons(2), especially to a quick brief stretch or "pluck," by a phasic response (jerk) there has not been forthcoming evidence of a static or maintained response by them to continued stretch. Nevertheless, they may play an important part in progressive movements by inhibiting the extensor's activity of stretch towards the end of the extensor phase of the step(6, 10 15a) It is known that tension applied to

a flexor muscle is able to inhibit the stretch-reflex of a corresponding extensor (10) This inhibitory action has been observed also in the present series of experiments A brief stretch or "pluck" applied, for example, to the tendon of the tibialis anticus muscle is well able to inhibit to resting paralytic level the stretch response of the gastrocnemius muscle during its kinetic recruiting stage or during its static tonic stage (Fig 1, *a, b, b*)

As concerns the possibility of a flexor muscle such as the tibialis anticus being itself able in the spinal condition to maintain reflexly a steady tonic level of tension in response to continued stretch, our condition of experiments have not shown that any such reflex is elicitable The myogram traced by the tibialis anticus muscle before denervation in response to a slow stretch (5 mm in 1 sec) differs little, if at all, from that traced by the muscle after denervation The maintained or tonic stretch-reflex is, in fact, according to our findings, absent from the flexor muscle in the chronic spinal condition In one animal, however, there was a big response to a quick pluck on the tendon ("pluck reflex"), which must be taken as evidence of a response to kinetic stretch, and is an observation in agreement with previous workers in this field

III In one preparation which of the series best showed the phenomenon of reflex walking at clinical examination, observations were made also on the vastocrureus muscle In the clinical examination before the experiment, resistance to flexion of the knee was definite and maintained sufficiently to support the animal's weight, but was not so extreme in degree as to resist a passive flexion somewhat greater than the animal's weight, that is, the "lengthening reaction" was present and easy of elicitation

At experiment, it was found to give a reflex response only in response to a quick stretch of 4 8 mm applied during 140σ but not to the same stretch during 350σ In the former case, there was a briefly maintained plateau, lasting some 120σ This was even less than might have been anticipated from the previous clinical examination, but may well be accounted for by impairment arising from the unavoidable irritation and exposure of skin edges deteriorating an already feeble reflex In this connection even the well-marked reflex response of the gastrocnemius muscle to stretch we found suffer from any but the most delicate handling of skin wounds in the stimulation of nerves On one occasion, for instance, mere exposure of the nerve and the application of electrodes (without electrical stimulus) diminished the reflex stretch response of the gastrocnemius muscle from a tension of 1 3 Kg to 0 8 Kg (see also

Fig 1) Although we have not sufficient evidence to settle the point, our definite impression is that, on the whole, the vastocrureus (an extensor) does give, after spinal transections between 2nd and 3rd post-thoracic segments, a briefly maintained response to the kinetic period of the stretch which resembles the response of the tibialis muscle (a flexor) under similar conditions The knee-jerk response to a tendon tap of the vastocrureus muscle in the experiment described had a duration, measured from the onset to the "angle" of $95\,\sigma$, which is of the same order as the duration of response of $120\,\sigma$ to the quick fall of the table-top

Summary and Conclusions

1   The effect of stretching the tendon of the gastrocnemius muscle (ankle extensor) with small maintained stretches within physiological limits has been examined in dogs, whose spinal cords have been transected at the level of the 1st–2nd or 2nd–3rd post-thoracic segments 2–3 months previously and allowed to recover from "spinal shock" A tension develops in the muscle which can be inhibited down to the tension of the paralysed muscle under a similar degree of stretch The inhibition may be brought about by stimulation of an appropriate afferent nerve or by stretching gently the tendon of an antagonistic muscle, tibialis anticus (ankle flexor)   When not inhibited the plateau of super-added tension continues as long as the stretch is applied ("tonic" or "static" stretch reflex)   It is concluded from these data that the maintained stretch reflex exists fundamentally as a spinal process which can be elicited from the spinal cord without the intervention of medullary or mid-brain centres This brings the stretch reflex into correspondence with the knee-jerk ("kinetic" or "phasic" stretch reflex) as a spinal phenomenon, which latter, as is well known, can be elicited during the existence of "spinal shock"

2   A well-maintained stretch reflex from the vastocrureus muscle has not been elicitable in our preparations (level of spinal section, as above)

3   A maintained reflex was not elicitable from the ankle flexor (tibialis muscle) (level of section, as above)

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ADRENIN AND THE SPLANCHNIC NERVE

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IT is well known that when the peripheral end of the splanchnic nerve in an animal under an anæsthetic is stimulated, the blood-pressure curve which one obtains is not a simple rise The initial rise of pressure is followed by a "dip" succeeded by a return to a level exceeding the initial height of the blood-pressure, which height is maintained as long as stimulation is continued This "dip" does not occur after elimination of the adrenal bodies The literature of this subject up to 1924 is given by Vincent and Wright(1), who considered that the "dip" is due to the pouring out of minute doses of adrenin into the bloodstream when the splanchnic nerve is stimulated

The discovery that very small doses of adrenin produce a fall of blood-pressure instead of a rise was made by Moore and Purinton in 1900 (2)   These authors worked with dogs under chloroform The same result is easily obtained in animals under ether anæsthesia

MacDonald and Schlapp(3) have recently demonstrated that this well-known depressor effect of small doses of adrenin is not an essential and constant action of the drug in such doses, but is due to a pharmaco-dynamical influence of the ether employed as anæsthetic We have been able fully to confirm these observations MacDonald and Schlapp affirm that this effect is marked under ether and urethane, less marked under chloralose if no ether has been previously administered We are able to add that the effect is readily observable when the animal is under chloroform There seems to be an optimum degree of anæsthesia at which the depressor effect is obtained Degrees of anæsthesia lighter or deeper than this result in a purely pressor effect

The initial level of the blood-pressure is a matter of fundamental importance It has been pointed out by many observers, though it is frequently forgotten, that when the pressure is very low one rarely gets a depressor response under any conditions

In reading the communication of MacDonald and Schlapp it occurred to us that if the "dip" in the splanchnic curve be, in fact, due

to the pouring out of small doses of adrenin, it would not be observed
in the absence of the anæsthetics above mentioned. We have accordingly
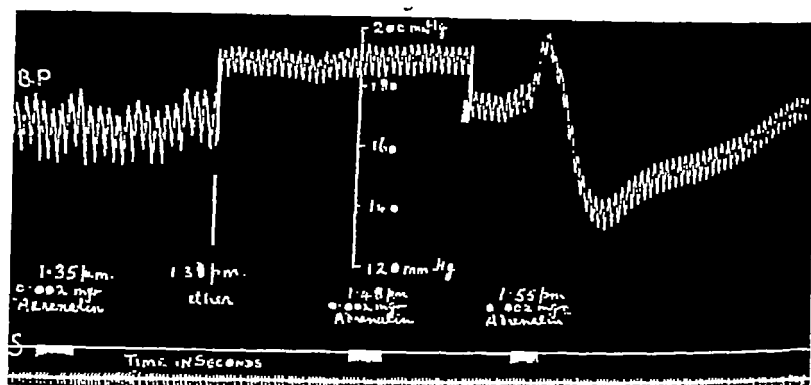
Fig 1   Cat ether   Decerebrated 10 45 a m   B P = carotid blood pressure   S = signal
Animal allowed to blow-off ether until 1 38 p m. Before this injection of 0·002 mgr
adrenalin produced no effect. Note that 10 minutes after ether is administered there
is still no sign of vaso dilatation, which, however, is very marked 7 minutes later

carried out a number of experiments upon pithed and decerebrate cats
and the uniform result has been that, when adrenin injected intra-
venously caused a vaso-dilatation, then and then only could we obtain
the "dip" in the splanchnic curve

It thus appears probable that in the normal intact animal adrenin,
even in the smallest doses, would tend to constrict the arterioles and
raise the blood-pressure

From the time of the discovery of Oliver and Schafer(4) that the
chromaphil tissues contain a pressor substance, it has been generally
assumed that one of the functions of the adrenal bodies is to help in
maintaining the normal blood-pressure   One of the arguments used
against this theory has been that any such quantities of adrenin which
could be normally passed into the circulation from the adrenal bodies
would tend to lower the blood-pressure and not to raise it   The observa
tions of MacDonald and Schlapp have removed the foundation of
this argument

It must, however, be remembered that there are other and very
strong objections to the tonic theory   The fact that extirpation of one
gland and denervation of the other (Stewart and Rogoff(5)) results in
no apparent difference in the health of the animal, even though the
adrenin output is reduced below detectable limits, indicates that adrenin

itself does not play any important part in maintaining the body functions under normal conditions Tying off or extirpating the organs does not
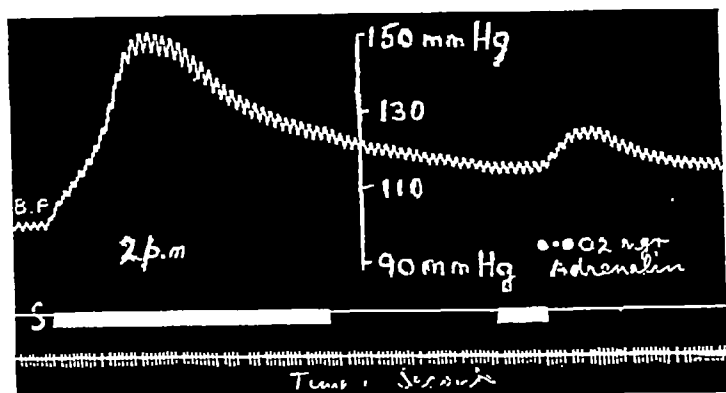
Fig. 2 a.
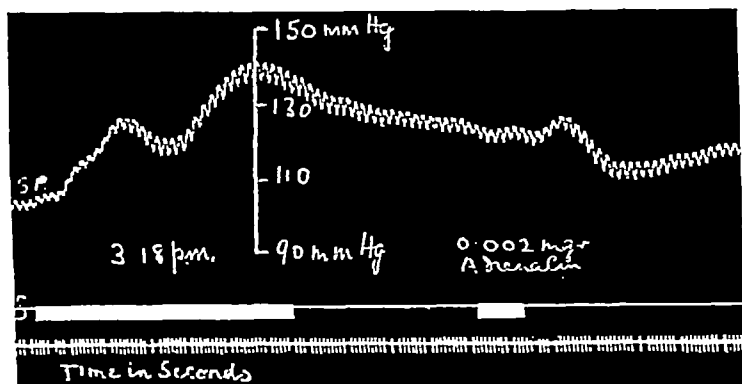
Fig 2 b

Fig 2 Cat ether Decerebrated 11 a.m Posterior dissection of left splanchnic nerve. B P = carotid blood pressure S = signal

(a) Stimulation of splanchnic nerve followed by an injection of adrenalin. Between 2 4 and 2 57 p m. ether was given. (b) Stimulation of splanchnic nerve followed by an injection of adrenalin

Note that in the decerebrate animal without anæsthetic there is a simple rise of blood pressure on splanchnic stimulation and a rise of pressure on injection of adrenalin, while after the administration of ether the splanchnic curve shows a "dip" and the injection of adrenalin produces a fall

appreciably affect the level of the blood-pressure (Young and Lehmann[6], Young[7], Austmann, Halliday and Vincent[8]) It

seems clear that it is the cortex and not the medulla which is essential to life (Biedl(9), Wheeler and Vincent(10))

SUMMARY

1   Minute doses of adrenin injected into the circulation produce a fall of blood-pressure instead of the customary rise which occurs with larger doses, but this fall occurs only when the animal is under the influence of certain anæsthetics   If, in pithed or decerebrate animals, the anæsthetic be eliminated, the depressor effect can no longer be observed (confirmatory of MacDonald and Schlapp)

2   The anæsthetics which are responsible for these effects, are ether, chloroform, chloralose, urethane and possibly others

3   It seems likely that small quantities of adrenin normally secreted by the chromaphil tissues, if they produce any effect at all on the circulation, might tend to keep the blood-pressure at its normal level

4   The "dip" in the blood-pressure curve which results from stimulation of the splanchnic nerve is, like the fall of pressure which occurs after minute doses of adrenin, dependent on the presence of one or more of the above anæsthetics   When they are eliminated in the pithed or decerebrate animal, the "dip" disappears and the splanchnic curve is a simple rise   Under the influence of other drugs there is a "hump" or secondary rise instead of a "dip" on the splanchnic curve   The "dip" and the "hump" in the splanchnic curve are thus due to minute quantities of adrenin poured out under the special pharmaco-dynamical conditions of anæsthesia, for they do not occur when the adrenal bodies are eliminated

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THE PHYSIOLOGICAL SIGNIFICANCE OF "PHOSPHAGEN"

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IN a recent publication[1] we have described methods for the detection and estimation in muscle tissue of an organic phosphoric acid derivative hitherto undescribed. This substance is of the nature of a hexosephosphate (unpublished results) It has previously escaped observation by virtue of two remarkable characteristics it is hydrolysed with great rapidity in acid solution, yielding inorganic phosphate, hence the estimation of inorganic phosphate by the methods of Embden[3] or Briggs[2] (which involve the use of strong mineral acids) leads to its complete destruction, and the estimation of its phosphoric acid radicle as "inorganic", in the second place, incubation of a chopped muscle in the presence of fluoride leads to the conversion of this substance into some acid-stable form For this reason Embden failed to detect it in his method for isolating the hexosephosphates of muscle[12], inasmuch as the first stage of his preparation was an incubation with fluoride (to increase the yield of "lactacidogen ')

This substance has been designated "phosphagen," since it appears to be the precursor of inorganic phosphate in the muscle It should not be confused with Embden's "lactacidogen," which is very resistant to hydrolysis by mineral acids in the cold. Phosphagen appears to be of special importance to the functioning of voluntary muscle The muscular lining of the frog's stomach contains so little that we cannot estimate it The heart muscle contains a little phosphagen, but is hardly comparable in this respect with skeletal muscle (Table I)

TABLE I.

	Stomach	Heart	Gastrocnemius
Inorganic phosphate	20	20	25
Phosphagen	0	5	55

The results are given as mg of phosphorus per 100 grm. of tissue In this, as in other tables the phosphagen figures refer to the "labile" phosphorus of the molecule.

We have examined the behaviour of phosphagen in muscular con traction and recovery, using for the purpose the gastrocnemius muscle of the frog (*R temporaria*) Fatigue induced by a 2–4 minute tetanus results in a disappearance of the greater part of the phosphagen of the muscle, with the simultaneous production of inorganic phosphate, lactic acid, and a substance (or group of substances) as yet unidentified, which contains phosphoric acid in an acid-stable combination Table II gives the results of some experiments of this type

TABLE II.

Experiment (nature of tetanus)	Phosphagen			Inorganic phosphate			Lactic acid		
	Before	After	Fall	Before	After	Rise	Before	After	Rise
1½ min. in air, direct stimulation, secondary coil at 12–10 cm	40	17	23	38	48½	10½	38	130	92
2 min. in air, stimulation through nerve, 0 cm.	59½	24	35½	28	49	21	—	—	—
2 min in N₂, direct, 24–10 cm.	47	17	30	33	51½	18½	87	190	103
3 min. in air, stimulation through nerve, 10 cm.	55	25½	29½	25½	45½	20	96	194	98
3 min. in air, stimulation through nerve, 0 cm	55½	21½	34	27½	46½	19	—	—	—
4 min. in air, stimulation through nerve, 0 cm	56	23	33	28	45	17	—	—	—

Effect of rapid fatigue The primary circuit of the stimulating coil contained one 2 volt accumulator (Results are given as mg per 100 grm of muscle, of phosphorus and lactic acid respectively)

It will be seen from these results that the disappearance of 30 mg of phosphagen-phosphorus is accompanied by the appearance of about 20 mg of inorganic phosphorus and about 100 mg of lactic acid These figures are suggestive of the glycolysis of a hexosediphosphate, accompanied by the re-synthesis of part of the inorganic phosphate into some compound other than phosphagen Even so, the lactic acid production is slightly excessive, but the discrepancy might be attributable to some cycle leading to the re-formation of phosphagen, and the subsequent breakdown of this, with the production of more lactic acid Some support is given to this view by the results in Table III In these experiments the tetanus was broken up and spread over a longer time in order to facilitate any such anaerobic cycle The result was a much greater pro duction of lactic acid, with very little alteration in the amount of phosphagen used up or the amount of phosphate liberated

TABLE III.

Experiment	Phosphagen			Inorganic phosphate			Lactic acid		
	Before	After	Fall	Before	After	Rise	Before	After	Rise
10 sec. tetanus, 30 sec. rest (each 15 times), 2 volt, 10 cm. indirect	53	21½	31½	23	51	28	60	256	196
5 15 30, 2 volt, 14 cm. direct	50	10	40	29	60	31	66	256	190
5 15 36, 2 volt, 10 cm. direct	37	2	35	41	69	28	57	263	206

Effect of discontinuous stimulation (in nitrogen)

It could certainly be argued that the lactic acid production is a process independent of the phosphagen disappearance, but the work of Meyerhof(4), on the fermentative properties of muscle extracts, leads one to suspect a connection. There can, however, be little doubt as to the intimate relationship between the inorganic phosphate and phosphagen. If a fatigued muscle (tetanised for about 3 minutes in nitrogen[1]) is exposed to an atmosphere of oxygen, it rapidly regenerates the phosphagen which had been lost during the tetanus. At the end of an hour most of the phosphagen has been replaced, *and an exactly equivalent amount of inorganic phosphate has disappeared.* During this time only a very small amount of lactic acid is removed. The results of five such experiments are quoted in Table IV.

TABLE IV

Experiment	Phosphagen			Inorganic phosphate			Lactic acid		
	Before	After	Rise	Before	After	Fall	Before	After	Fall
30 min. at 16°	5	22½	17½	70	52½	17½	—	—	—
60 „ 16°	11	32	21	62	41	21	194	169	25
40 „ 4°	6½	41½	35	65	30	35	—	—	—
50 „ 4°	7	46	39	65	26½	38½	—	—	—
60 „ 4°	15½	36½	21	55	35	20	164	152	12

Effect of recovery in oxygen. The muscles had been tetanised for 2–4 minutes in N_2

Although a comparison of Table III with Table II leads one to suspect the existence of a cycle involving glycogen, phosphate, and phosphagen in the production of lactic acid, yet we have found no direct evidence of any anaerobic recovery leading to an increase in the phosphagen content of a muscle. Whether resting or fatigued, a muscle placed in nitrogen always tends to lose its phosphagen (Table V)

[1] The nitrogen used in all our experiments contained about 1 p.c. of oxygen. In view of the recent work of Furusawa and Hartree (5) this fact may subsequently assume importance

TABLE V

Experiment	Phosphagen			Inorganic phosphate		
	Before	After	Fall	Before	After	Rise
Resting muscle (18 hours 16°)	(55)	10	(45)	(25)	63	(38)
Fatigued muscle (1 hour 16°)	16	7½	8½	64½	70½	6
Fatigued muscle (1 hour 16°)	15¼	6	9¼	59	77	18

Effect of anaerobiosis on resting and fatigued muscles The initial condition of the resting muscle was not determined average values have been inserted. In this experiment the lactic acid rose to 535 mg p c

Experimental

The gastrocnemius muscle of the frog (*R temporaria*) was used throughout this work The muscle was dissected away from the bone, with the sciatic nerve attached if required We have satisfied ourselves that the slight injury necessitated at the origin of the muscle by this technique has no serious effect on its subsequent behaviour Where it was desired to obtain a close approximation to the theoretical "resting condition," the frogs were previously cooled for a few hours in a room at 0° Stimulation (either direct or *via* the nerve) was applied through platinum electrodes from a small induction coil set to give a maximal stimulus (as judged by the response of the muscle, which was always allowed to contract freely) It was found convenient, though not essential, to use liquid air for killing the muscles Where one gastrocnemius was used as a resting control on its companion, it was maintained under the same conditions of temperature and atmosphere as its stimulated companion, and the two were immersed simultaneously in liquid air

In cases where lactic acid was estimated, batches of four to six frogs were used in each experiment, but in most other cases the measurements here recorded were made on the muscles of a single frog

In testing the effect of recovery in oxygen, the muscle pairs were stimulated in parallel on the electrodes One muscle was immediately killed in liquid air, and the other suspended in moist oxygen at a pressure of 80–100 cm of mercury

The removal of proteins was in all cases effected by grinding up the tissue in 4 p c trichloracetic acid The method of estimating phosphagen and inorganic phosphate has been outlined in a different publication[1] It is a modification of the Briggs technique, which in its ordinary form does not discriminate between the phosphagen and inorganic phosphate, owing to the rapid hydrolysis of the former Lactic acid was estimated by a modification of the Clausen technique[6], suggested to us by Prof Meyerhof This modification is based on the fact that no aeration is necessary in the Clausen distillation if certain precautions are taken

Discussion

There is present in the skeletal muscles of the frog an organic phosphorus compound which has hitherto been confused with inorganic phosphate, owing to its rapid hydrolysis in acid solution to phosphoric acid There may be more than one such compound, but the hypothesis of a single compound is sufficient to explain the available facts   We have given the name "phosphagen" to this substance

The results quoted in this paper establish the fact that muscular contraction is accompanied by the removal of phosphagen, and subsequent recovery in oxygen is characterised by a rapid restitution of the phosphagen—a phase of recovery apparently independent of the relatively slow oxidative removal of lactic acid (7)   It is of interest to consider the possible relationship between phosphagen, inorganic phosphate, and lactic acid   But it must be remembered, in the first place, that we can estimate phosphagen only in terms of its exceedingly labile phosphate radicle the molecule may contain phosphate radicles more resistant to acid hydrolysis   In the second place, we have no knowledge of the nature of the organic part of the molecule[1]   Out of the several possibilities we put forward the following scheme, which has the advantage of simplicity

(*a*) Phosphagen $\rightarrow$ Lactic acid $+$ Inorganic phosphate

(*b*) Inorganic phosphate $+$ Glycogen $\rightarrow X$

(c) $X \rightarrow$ Phosphagen

Reference to Table II shows that part of the labile phosphagen-phosphorus which disappears in fatigue is not accounted for by the inorganic phosphate which is liberated. In the scheme above this unidentified product has been labelled X, and is represented as being formed at the expense of inorganic phosphate, and not directly from phosphagen X may be identical with Embden's lactacidogen These three reactions form a cycle of changes, which, if properly balanced, leads simply to the conversion of glycogen into lactic acid   By imagining one of these reactions to be temporarily accelerated, as by electrical stimulation or changes in the oxygen supply, the observed effects can be duplicated

It is evident that this view of the function of phosphorus in the metabolism of striated muscle affords an independent confirmation of the conclusions of M e y e r h o f (4), in his recent studies of the fermentation processes in muscle extracts

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[1] Recent unpublished work on the isolation of phosphagen shows that it is a hexosemonophosphoric acid, though some doubt attaches to the nature of the hexose

It is premature at this stage to review the existing literature in the light of this new discovery, but it is necessary to emphasise the fact that all work based on the methods of Briggs(2) or Embden(3), involving the use of strong mineral acid for the estimation of phosphate, becomes of doubtful validity We have found phosphagen in the skeletal muscles of the tortoise, rabbit and guinea-pig, and from an examination of the work of Beattie and Milroy(8), Andrews(9), and others, we are strongly inclined to suspect the presence of phosphagen in the muscles of cats and dogs The sudden increase in the inorganic phosphate content of the blood of athletes during a short severe spell of exercise(10), and the increased excretion of phosphate under similar conditions(11), becomes easily explicable on the assumption of the presence of phosphagen in human muscle

The "synthetic" effect of fluoride on minced muscle will be the subject of a separate communication, but a passing reference will be of interest Phosphagen disappears from a bicarbonate suspension of minced frog muscle, whether fluoride is present or not but in the former case it gives rise to no inorganic phosphate It is only necessary to suppose that fluoride completely inhibits reaction (c) in our scheme in order to explain these facts In the absence of fluoride the complete cycle can be effected, though reaction (a) obviously predominates

SUMMARY

1   There is present in the gastrocnemius muscle of the frog an organic derivative of phosphoric acid, hitherto undescribed, which takes a part in the chemical mechanism of contractility This substance has been designated "phosphagen "

2   The amount of this substance in a resting gastrocnemius, measured in terms of its labile phosphorus, is about 55 mg p c In the cardiac muscle there is only about one-tenth of this amount in the muscular coat of the stomach none has been detected

3   A gastrocnemius tetanised for 2–4 minutes electrically (either directly or through the nerve) loses most of its phosphagen About two-thirds of the labile phosphorus which has disappeared is accounted for by the appearance of inorganic phosphate The simultaneously produced lactic acid bears a ratio to the phosphagen phosphorus lost of rather more than 1 molecule per atom of phosphorus

4   In a more slowly fatigued muscle (in nitrogen) the lactic acid production is considerably greater, whilst the phosphagen disappearance and the phosphate formation are about the same

5 No restitution of phosphagen can be observed under anaerobic conditions, but in the presence of oxygen phosphagen rapidly reappears, and an exactly equivalent amount of inorganic phosphate is lost During this time there is very little removal of lactic acid

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A SOURCE OF ERROR IN MEASUREMENT OF THE CIRCULATION RATE BY HENDERSON AND HAGGARD'S METHOD

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In this paper an attempt is made to subject the ethyl iodide method[1] to a more rigorous analysis than is at present to be found in the literature, and it shows that the results which the method gives may be 20 to 30 p c too large   A second error of quite a different nature is pointed out qualitatively, and makes it doubtful whether circulation rates deter mined by this method are proportional to those actually measured in pathological cases

When discussing the alveolar air in the lung at any given moment two problems at once arise

(1) Are the different lobes of the lung uniformly ventilated with regard to their blood supply and, if so, is the alveolar air of the same composition at the apex and base and everywhere else?

(2) Is the air in the atrium and its air-sacs, in other words, in the single alveolar group, completely mixed?

Krogh and Lindhard[2] have put forward evidence to show that in normal individuals the answer in both cases is "yes," and that the error due to unequal ventilation is, in both cases very small   The paper is divided into two parts, in the first these two assumptions have been made and are supported by the data to which reference has already been made

I   By taking mechanical samples of alveolar air at different *known* times during a normal expiration Krogh and Lindhard[3] have deter-mined the relation between the tension of carbon dioxide and oxygen in the lung and the time during the expiratory phase of the respiratory cycle   A curve for carbon dioxide tension, together with a curve for the spirometer readings in the same experiment, is here reproduced from their paper (Fig 1)   Krogh and Lindhard assumed that during inspiration the tension of carbon dioxide *falls off to the initial value in a straight*

lıne This assumptıon cannot be supported by experımental evıdence, as ıt ıs not possıble to take alveolar samples durıng ınspıratıon, but a calculatıon to follow shortly wıll show that thıs assumptıon ıs not far from the truth, but thıs ıs not always the case
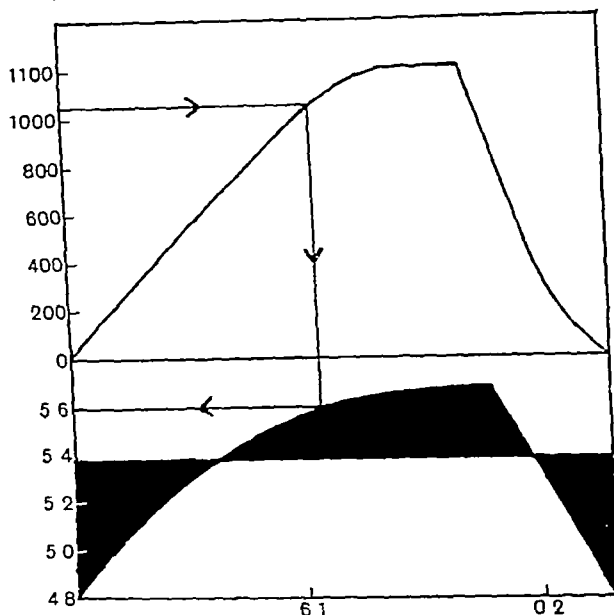
Fıg 1 Above spırometer readıngs ın c.c. Below percentage of carbon dıoxıde ın the alveolar aır Tıme ın mınutes as abscıssa

Suppose now that the subject ın Krogh and Lındhard's experı-ment ıs breathıng through a Henderson mechanıcal sampler for alveolar aır, ın whıch the last few c c of each expıratıon (10 or less) are collected, and that he ıs breathıng ın exactly the same way as ın Krogh's experı-ment, ıt wıll then be possıble to read off on the graph the tensıon of carbon dıoxıde ın the mechanıcal sample ın the followıng way The dead space of the subject J L , together wıth the mouthpıece used, has been accurately determıned as 150 c c , and therefore the aır whıch ıs ın the sampler wıll be ın the lung of the subject when 150 c c stıll remaıns to be expıred The tıme at whıch the last 150 c c ıs stıll to be expıred ıs read off from the spırometer curve and the alveolar carbon dıoxıde tensıon at thıs tıme wıll be the tensıon ın the sampler (5 6 p c CO_2) The average tensıon or percentage of carbon dıoxıde can be found from the curve by a graphıc method, and ıs shown by a lıne parallel to the abscıssa drawn ın such a way that the area enclosed by the part curve

above it is equal to the sum of the areas between it and the curve at each end These are drawn in black in the diagram and show that for this curve the average alveolar percentage will be 5 38 p c carbon dioxide It will at once be seen that the average alveolar tension and the tension in the Henderson mechanical sampler are different and that if the venous pressure and CO_2 output for the subject were known the calculation of the circulation rate by the Fick method for carbon dioxide would be different if one took as values for the arterial carbon dioxide tension the average alveolar tension or, on the other hand, the tension in the Henderson sample The following table, taking normal but fictitious values for the venous tension and the output, shows the order of magnitude of this difference for this experiment and also for another in which Krogh and Lindhard give the carbon dioxide curve for the subject doing work on a bicycle ergometer

TABLE I. Circulation rate calculated by the Fick method from carbon dioxide data
The arterial carbon dioxide tension determined by the Henderson method.

	From "average alveolar" tension	From tension during expiration of last 10 c c.	Error
Rest	6 litres	7 2 litres	20 0 p c.
Work	15	20 2	34 6

In the case of the work experiment the spirometer curve was not given, and the dead space could not be allowed for, but it is probable that even if this was taken into account the order of the error would not be changed very much

There is one other assumption in this calculation which is not justified, and it is that the average alveolar tension for the time curve is the same as the average tension to which the blood is exposed This would only be true if the blood flow through the lung during respiration was perfectly regular, which is not probable, only if the tension of carbon dioxide at different times was plotted against the fluctuations in blood-flow and the average of this curve taken would the true average alveolar tension to which the blood was exposed be obtained

Further work by Krogh and Lindhard(4) gives data for calculating not only the fluctuations in the blood-flow through the lung during a single respiration, but also for calculating the actual curve for the ethyl iodide tension in the lung during any of their experiments Hence for seeing how far the circulation rate obtained by taking the Henderson mechanical sample would differ from the real circulation rate calculated from the average tension of ethyl iodide to which the blood was exposed

Three of these calculations were made, one is given here in detail together with the conclusions drawn from the other two

The data taken from the first of K r o g h 's rest experiments and used in the calculation are given in Table II

<div align="center">TABLE II.</div>

Time from beginning of expiration	Spirometer reading c.c	Oxygen intake c.c	Carbon dioxide output c.c.	Initial values	Inspired air
0·408	440	8·5	16·6	Lung vol. 4140 c.c	—
1·070	1020	32·7	29·0	O₂ p c. 15·8 } in alveolar air	O₂ 21·79 p c.
1·770	1020	48·9	31·5	CO₂ p c. 4·8 }	CO₂ 0 p c.
2·320	0	67·9	52·8	Dead space 150 c.c.	
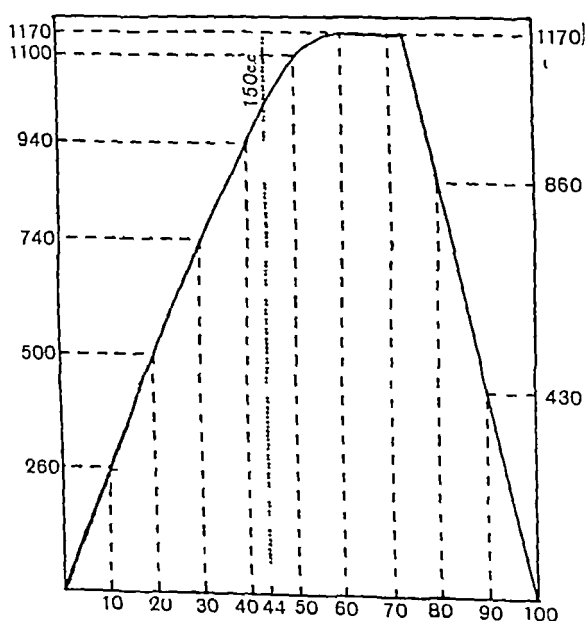
Fig 2 Spirometer readings Abscissa time as a percentage of the total time for the cycle
Ordinates show the spirometer readings in c c. at the end of each of the ten periods.

Curves for the spirometer readings (Fig 2) for the oxygen intake and the carbon dioxide output (Fig 3) are drawn directly from the table The spirometer reading is given in Table II as 1020 c c at two consecutive times and between these two times the dead space is expired and again inspired, this part of the curve is extrapolated and as the general form of the curve is well known the errors will not be very large,

the dead space of the subject had been previously accurately determined
as 150 c c
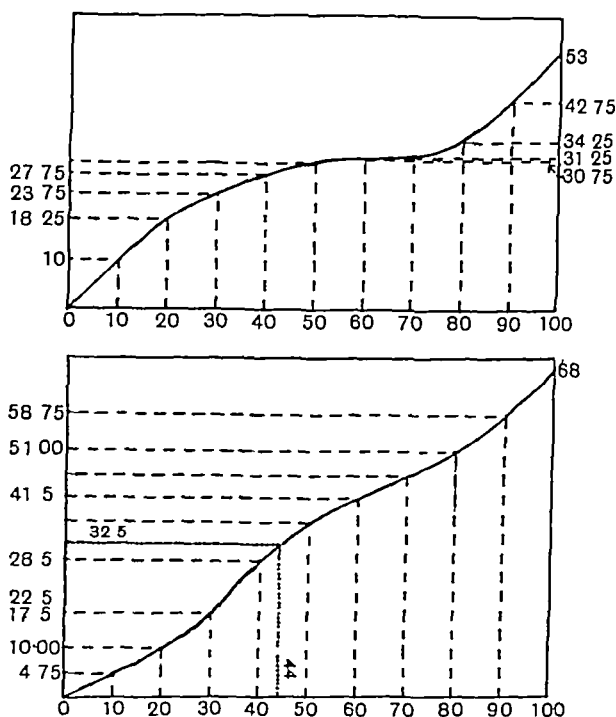
Fig 3   Abscissa   time in percentage of the total time for the cycle. Above—ordinate
c c. of carbon dioxide given off in the lung from the blood. Below—ordinate c.c of
oxygen absorbed by the blood in the lung

The time is expressed as a percentage of the total time, 0 232 minute,
and each abscissa is divided into ten equal parts   Knowing these three
curves, and the fact that the volume of the lung at the commencement
of the expiration was 4140 c c , the volume of air in the lung at the
end of each of the ten periods is calculated   It is the volume at the
beginning of the period + or − the tidal air, + the carbon dioxide
added from the blood during the period, − the amount of oxygen
absorbed into the lung during the period   Thus the volume at the end
of the first period will be

4140 − 260 (air expired) + 10 (CO_2 added) − 5 (O_2 absorbed) = 3885 c c

These values are shown in Table III in the first five columns

The percentages of oxygen and carbon dioxide in the alveolar air at
the start are also given and the initial volume of air in the lung is

TABLE III.

Time from start in percentage of total	Air added or subtracted by breathing from Fig 2	Oxygen absorbed in each period from Fig 3	Carbon dioxide added from Fig 3	Lung volume at end of period	Oxygen removed or added in breathing	Total volume of oxygen in lung	Per centage oxygen in lung	Carbon dioxide left or added in breathing	Total volume of carbon dioxide in lung	Percentage carbon dioxide in lung
0	0	0	0	4140	0	655	15 8	0	198 5	4 8
10	−260	− 4 75	+10	3385	−41	610	15 7	−12 5	196	5·05
20	−240	− 5 25	+ 8 25	3647	−38	567	15 55	−12 0	192 25	5 275
30	−240	− 7 25	+ 5 5	3405	−37	522 5	15 35	−12 5	185 25	5·435
40	−200	−11·0	+ 4·0	3198	−31	480 5	15·02	−11·0	178 25	5 575
50	−160	− 8·0	+ 2 25	3032	−23	449 5	14 82	− 9·0	171 25	5·65
60	− 70	− 5·0	+ 0 75	2958	−10 5	434·0	14·65	− 4·0	168·0	5 68
70	0	− 4 5	+ 0 5	2954	0	429 5	14 53	0	168 5	5 71
80	+310	− 5·0	+ 3 25	3266	+22·0 D +35·0	481 5	14 75	+ 7 5 D 0	179 25	5·50
90	+430	− 7 75	+ 8 25	3696	+93 5	567 5	15 33	0	187 25	5·075
100	+430	− 9 25	+10·25	4127	+93 5	632·0	15 80	0	197 75	4 81

known, so the total quantity of carbon dioxide and of oxygen present in the lung at the beginning of expiration is known, these are shown in the first row of figures in the table The quantity of oxygen and carbon dioxide in the lung at the end of each of the ten periods can be calculated in exactly the same way In the case of oxygen it will be the initial quantity + or − the quantity in the tidal air, − the quantity absorbed by the blood The only difference in the carbon dioxide calculation is that the quantity of carbon dioxide leaving the blood is added to the initial volume instead of subtracted Thus the volume of oxygen in the lung at the end of the first period will be 15 8 p c of 4140 = 655 (initial volume) − 4 75 (absorbed) + 15 8 p c of 260 = 41 (entered with tidal air), and the final figure is

$$655 - 4 + 41 = 610 \text{ c c}$$

The ratio of the total quantity of oxygen or CO_2 left in the total volume of the lung at the end of the period gives the percentage of the gas at the end of the period, and for oxygen in the example above is $\frac{610}{3385} \times 100 = 15\ 7$, these percentages are given in columns 8 (O_2) and 11 (CO_2) All the data are now available for the calculation of the total quantities of carbon dioxide and oxygen in the lung volume at the end of the next period and so on till the end of expiration. At the start of inspiration the dead space will be re-breathed and this is allowed for thus in period 8 the first 310 c c of inspired air enter the lung— this is composed of 150 c c dead space air and 160 c c inspired air The dead space air is assumed to have the same composition as it had when it left the lung in the previous period, in this case for oxygen 14 53 p c and for carbon dioxide 5 71 p c Values for the composition

of the inspired air used in the experiment are to be found in Table II
The percentages of oxygen and carbon dioxide in the lung at the end
of the different periods have been plotted in Fig 4 and agree well with
the values found by experiment by taking automatic samples at known
times, as can be seen from the curves
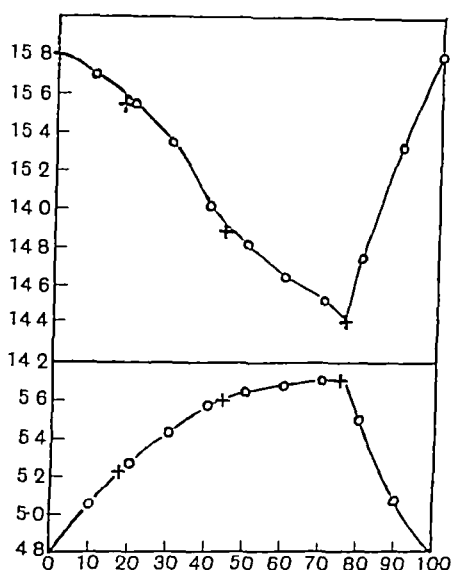
Fig 4 Abscissa time as a percentage of the total time for the cycle Above—ordinate
percentage oxygen content of the alveolar air Below—ordinate percentage carbon
dioxide content of the alveolar air

O Calculated. + Experimental values, Krogh and Lindhard.

Nothing more is claimed for this calculation than this, that the
agreement between the actual and calculated values for the percentages
is close enough to establish the fact that for this particular experiment
(and not necessarily for any other) the ratios of the total quantity of
carbon dioxide or oxygen left in the lung at the end of the different
periods to the lung's volume at the end of these periods is correct for
these give the points on the curves directly If this be admitted it
follows that, as the volumes for the lung at the end of the periods are
taken directly from the data and represent the actual volume of the lung
the tensions calculated must also be the actual tensions It is therefore
claimed that correct values for the quantity of nitrogen could be worked
out for this experiment with equal accuracy, or if any other gas was added

in a quantity, which, compared to the total lung volume was negligible, its percentage curve could be worked out correctly

The concentrations of ethyl iodide for use in determining the circulation rate are made up using 0 3 c.c of liquid iodide per 100 litres of air, and so, assuming roughly this dilution for the quantity in each litre of air in the lung during an ethyl iodide experiment, 0 5 c c of iodide vapour per litre is approximately the amount in the lungs of a subject at the start of expiration, this is slightly on the high side The volume of this compared with the volume of air in the lung is quite negligible In this particular experiment 2 0 c c of ethyl iodide is assumed to be present in the lung of J L at the beginning of his expiration, and during the expiration it will be diminished by quantities which leave in the expired air and in the blood

By reference to the oxygen curve in Fig 4 it will be seen that the lowest oxygen percentage reached is 14 42, which represents a tension of 109 5 mm. of oxygen, and the highest is 15 8 p c , which represents a tension of 120 mm. Using as constants in Hill's formula for the relation between the percentage saturation of the blood and the tension on oxygen to which it is exposed $K = 0\ 0028$ and $n = 2\ 5$, it follows that for the highest pressure the blood will be 97 8 p c saturated with oxygen and 97 3 for the lowest, and the average about 97 6 This difference is so small that the saturation has been assumed to be constant at 97 6 p c , and as the venous pressure will also be constant the *blood-flow will be proportional to the oxygen consumption* This is so near the truth that, with the value for the circulation rate given below, the highest oxygen tension represents the quantity of blood flowing through the lungs during the first period as 0 59 p c too low, and the lowest tension, found in period 7, will represent it as 0 59 p c , the same quantity too high These errors have not been taken into account

Any total blood-flow for one respiratory cycle may be assumed for this calculation, but once fixed the oxygen venous pressure is fixed too Here the values taken are to be found in column 2 of Table IV and the total flow is 1360 c c , twenty times the oxygen consumption, similarly, the blood-flow during each period will be twenty times the oxygen consumption for the period The ratio of the concentrations of ethyl iodide in the alveolar air and in the blood at any time is determined by a distribution coefficient K, which has here been taken as 2 The quantities of ethyl iodide leaving during the period in the expired air (alv p c × air expired, Table IV, column 5) and in the blood (alv p c × K × blood-flow, Table IV, column 4) are calculated in just

TABLE IV

Time	Blood flow	$K \times$ blood flow	Quantity of C_2H_5I absorbed by the blood	Quantity added or taken away by breath	Total quantity in the lung	Percentage C_2H_5I in lung	Percentage blood flow
0	0	0	0	0	2 000	0484	0
10	95	190	− 092	− 126	1 782	0459	7·0
20	105	210	− 096	− 110	1 576	0432	14 7
30	150	300	− 130	− 104	1 342	0395	25 8
40	220	440	− 174	− 079	1 089	0341	41 9
50	160	320	− 109	− 054	0 926	0305	53 7
60	100	200	− 061	− 021	0 844	0285	61 1
70	90	180	− 052	0	0 792	0268	67 7
80	100	200	− 054	+ 040 D	1 010	0310	75 0
				+ 232			
90	155	310	− 096	+ 620	1 534	04155	86·4
100	185	370	− 154	+ 620	2 000	0484	100
Total	1360	Total	− 1 017	0·1445 p c. C_2H_5I in inspired air			

the same way as those of oxygen or carbon dioxide in Table III During inspiration the quantity in the inspired air is found by trial and error and is the amount that the inspired air must contain for the total amount of ethyl iodide in the lung at the end of inspiration to be the same
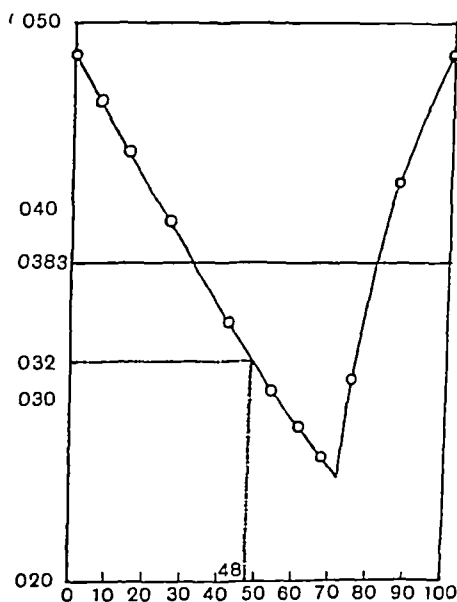
Fig 5 Abscissa blood flow as a percentage of the total blood flow for the cycle. Ordinate percentage of ethyl iodide in the alveolar air in the lung

as it was at the beginning of expiration 2 000 c c The curve for the percentage of ethyl iodide in the lung during this cycle is plotted in Fig 5, not against time, as in the previous curves, but against the percentage bloodflow (Table IV, col. 8) through the lung The average tension of ethyl iodide to which the blood is exposed is calculated graphically as before and is found to be 0 0383 p c Table IV, column 4, shows that the total quantity of ethyl iodide absorbed into the blood during the respiration is 1 017 c c and these data are sufficient to calculate the circulation rate for

$$\text{Circulation rate} = \frac{\text{quantity of ethyl iodide absorbed in given time}}{\text{distribution coefficient} \times \text{average tension during the time}}$$

$$= \frac{1 \cdot 017 \times 100}{2 \times 0 \cdot 0383}$$

$$= 1362 \text{ c c}$$

This checks excellently with the value 1360 c c which was the blood-flow assumed and is a credential for the accuracy of the calculation.

All the data are now here for reading off the circulation rate which would be given by the Henderson apparatus taking samples of the last 10 c c of expiration only Referring to Fig 2, the spirometer curve, it will be seen that when 150 c c , the dead space, has still to leave the lung (Fig 2, dotted line) 44 p c of the duration of the cycle has elapsed, 44 p c of the duration of the cycle represents an oxygen consumption of 32 5 c c (Fig 3, dotted line), and this is 47 8 p c of the total oxygen consumption, 68 c c , but the oxygen consumption is proportional to the blood-flow and the percentage of ethyl iodide in the Henderson mechanical sample shown by Fig 5, dotted line, is 0 032

Circulation rate for the cycle obtained by Henderson sampler $= \frac{1 \cdot 017 \times 100}{0 \cdot 032 \times 2}$

$= 1590$ c c

The actual circulation rate, however, was 1360 c c , and this is an error of 16 9 p c , and, correspondingly, the apparent circulation rate per minute will be 16 9 p c too high, 6 85 litres instead of 5 86

The summary of two other calculations, the second by a slightly different method, is given in Table V

TABLE V

Data from Table IV, p 269, *Biochemische Zeitschrift*, 59 1914.

Time in minutes	0·008	0·0213	0·0336	0·0421	—
Calculated oxygen percentages	17·08	16·22	15·29	14·6	—
Experimental oxygen percentages	16 96	16 14	15·29	14 6	—
Circulation rate assumed	16 120 litres per min.				
Average blood tension circulation rate	16 200			0·495 p.c.	Error
Henderson method circulation rate	19 950			23 7 p.c.	

TABLE V, *contd.*

Data from Table VII, p 270, *Biochemische Zeitschrift,* 59 1914.

Time in minutes	0 0089	0 0132	0 0242	0 0400	0·0478
Calculated oxygen percentages	15 14	14 85	14 13	12 42	12·03
Experimental oxygen percentages	15 17	14 87	14 13	12 42	12·03
Circulation rate assumed		19 730			Error
Average blood tension circulation rate		19 400		1 67 p c	
Henderson method circulation rate		26 400		33 7 p c.	

In both cases the error is very much larger, 23 7 p c and 33 7 p c Too much faith cannot be laid on the second calculation for the oxygen tension here drops below 100 mm (91 mm) for the end of expiration and the rebreathing of the dead space, and this fact renders it untrustworthy both for the present purpose and for the purpose it is used for in Krogh's paper[1] The calculated and experimental oxygen tensions, and also the circulation rate calculated from average blood tensions, are given in the tables as checks for the calculation

Some recent experiments of Prof Henderson's[5], in which the writer was a most interested assistant, show that the average quantity of ethyl iodide in Haldane-Priestley samples taken at the end of inspiration and expiration agrees very well with the concentration in the automatic sampler, and Henderson concludes that the mechanical sample represents the average tension of ethyl iodide throughout the respiratory cycle Krogh and Lindhard[6], however, show that the average of two Haldane-Priestley samples, one taken at the end of expiration and the other at the end of inspiration, is higher in CO_2 tension and lower in oxygen tension than the true average for those gases, the reason for this being that the taking of the Haldane-Priestley sample takes quite finite time and as the tensions of the gases are always varying rapidly with the time they will have changed during the taking of the samples

Each sample will contain less ethyl iodide than was present actually at the end of inspiration and expiration This explains the agreement Henderson finds between the automatic sampler tensions and the average of two "Haldane-Priestleys "

II   The second source of error lies in the fact that certainly in some pathological cases, and possibly in some normal cases, it may not be justifiable to assume that the ventilation is proportional to the blood supply The capillaries to some of the alveoli, or some of the capillaries to some of the alveoli, may be shut off On the other hand, some of the

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[1] Subsequent calculation, however, shows that at the time when the tension is highest the circulation rate given will be only 2 85 p c too low and for the lowest tension it will only be 1 49 p c too high

alveoli may be closed or damaged so that their ventilation is not the same as the other parts of the lung

Suppose that the capillaries to some of the alveoli are shut, and, taking an extreme case, suppose that the whole of the arterial supply to one lung is cut off The air inspired into this lung will not be able to get rid of any of its ethyl iodide, and during expiration this will be mixed with the ethyl iodide coming from the other normal lung, and the resulting mixture will contain more ethyl iodide than the alveolar air in equilibrium with the blood, hence the quantity of ethyl iodide absorbed is being divided by a quantity which is too great and the real circulation rate is faster

On the other hand, suppose that some of the alveoli are closed or filled with liquid Take an extreme case in which the bronchus of one lung is closed The alveolar air now obtained will be true alveolar air, but the quantity in the blood will be less than twice the quantity in the alveolar air, since the blood of one lung contains no ethyl iodide, again, the quantity of ethyl idodide absorbed is being divided by a quantity which is too great and the real circulation rate is bigger These two effects are additive

It seems, then, that one is forced to the conclusion that so valuable a clinical method as this cannot be regarded as sufficiently accurate for practical use, for, owing to errors in the calculation arising from the concentration in the automatic sampler not being the "average blood tension" and the fact that capillaries and alveoli may be closed, the apparent circulation rate may be anything from 25 p c larger to an indefinite quantity smaller than the actual

It is hoped when opportunity for experimental work arises, and if other difficulties(7,8) are overcome, to publish results for an ethyl iodide method which has the backing of the present subject matter

Summary

1 The curve for the variations in the tension of ethyl iodide during a respiratory cycle is calculated and a difference between the average tension of ethyl iodide to which the blood is exposed and the tension obtained in the Henderson automatic sampler is shown.

2 This difference may lead to the apparent circulation rate being 25 p c greater than the actual

3 Owing to the fact that capillaries and alveoli may be closed, possibly in normal and certainly in pathological cases, the apparent circulation rate may be too small

H BARCROFT

The writer is very much indebted to Professor K r o g h for kindly criticism

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THE ADJUVANT ACTION OF THE LACTATE ION ON THE VASO-DILATOR EFFECT OF SODIUM NITRITE

By BEN DENSHAM.

(From the Department of Physiology, King's College, London)

In the course of a recent paper by Hemingway and McDowall(3) on the acid-base control of the capillaries, it was pointed out by them that it was sometimes impossible to obtain the dilator effect of histamine in a perfused limb until a dose of lactic acid or acetyl choline had been administered   From this it appeared that the latter drugs exerted some sort of "unlocking effect" on the histamine dilator mechanism.

The present investigation was undertaken with a view to finding out whether a similar phenomenon exists in relation to the dilator effects of two other physiologically important drugs, namely sodium nitrite and acetyl choline

The method used was that of Sharpey-Schafer modified by McDowall(1), and fully described by Hemingway in a recent paper(2)   It consists of perfusion from a constant pressure reservoir, the perfusion pressure being registered directly by means of a side tube connected to a manometer and transmitted thence to a piston recorder

The perfusion fluids were Ringer's solution warmed to 37° C and buffered at varying cH's with sodium phosphate mixtures   All doses of drugs for injection were made up to 0 5 c c   The fore and hind limbs of cats were used exclusively

Hemingway and McDowall(3) showed that the loss of capillary tone in the perfused hind limb of a cat is due to the accumulation of lactic acid in the tissues   This can be obviated, as they showed, by neutralising the acid by perfusing with a fluid buffered at about pH 7 6 which is on the alkaline side of functional neutrality, or else, as shown by Dale and Richards(4), by oxidising the acid by perfusing with oxygenated Ringer s solution containing erythrocytes

It was considered expedient in the first place to discover whether the tone of the vessels on which acetyl choline and sodium nitrite act is to any extent under a similar acid-base control.

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It was considered expedient in the first place to discover whether the tone of the vessels on which acetyl choline and sodium nitrite act is to any extent under a similar acid-base control

This was found to be the case   If the perfusing fluid was of a pH of 7 4 or under (either buffered or unbuffered) there was a fall in the per fusion pressure and a disappearance of the vaso-dilator effects of acetyl choline and sodium nitrite   The speed at which the pressure fell, and the vaso-dilator actions disappeared, was greater the lower the pH of the perfusion fluid (pH 7 4–6 6)   If, on the other hand, the perfusion fluid was buffered at pH 7 5–7 6, the vaso-dilator effects of the two drugs persisted for 3–4 hours, at which point œdema generally precluded further work

The animals were either killed 5 minutes before the commencement of perfusion or else were prepared by the method of H e m i n g w a y and M c D o w a l l   This consists in giving repeated small (2 c c) doses of satu rated sodium bicarbonate followed by short periods of asphyxia to enable the alkali to enter the tissues   The injections must be intravenous and should be made about 1 hour before death

In such animals, when perfused with fluid buffered at pH 7 6–7 7, it was often impossible to obtain the vaso-dilator effect of either acetyl choline or sodium nitrite   Injection of 0 5 c c of $\frac{1}{100}$ lactic acid or $\frac{1}{20,000}$ hydrochloric acid produced a great fall in perfusion pressure with a rapid return to normal   After this, acetyl choline and sodium nitrite produced large vaso-dilations (Fig 1)
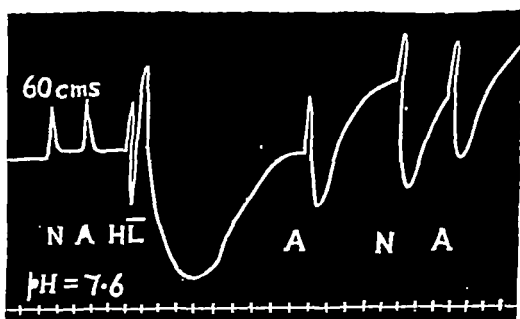
Fig 1   The ' unlocking effect   of lactic acid on the vaso dilator actions of acetyl-choline and sodium nitrite   HL=0·5 c.c. 1 p c. lactic acid injected.   N=0·5 c.c. 1 p.c. sodium nitrite injected.   A=0 5 c.c   01 p c. acetyl-choline   Perfusion pressure=60 cms of Ringer pH of entering fluid=7 6   Time tracing in minutes

It was noted in alkaline preparations as above, perfused with fluid buffered at pH 7 6–7 7, that if the nitrite and choline were injected, first after a dose of hydrochloric and then after one of lactic acid (the

doses of acid being such that they caused an equal fall in perfusion pressure), then a dose of nitrite which gave a smaller fall in pressure than a given dose of acetyl choline after the injection of hydrochloric acid, gave a much bigger one after the injection of lactic acid (Fig 2)
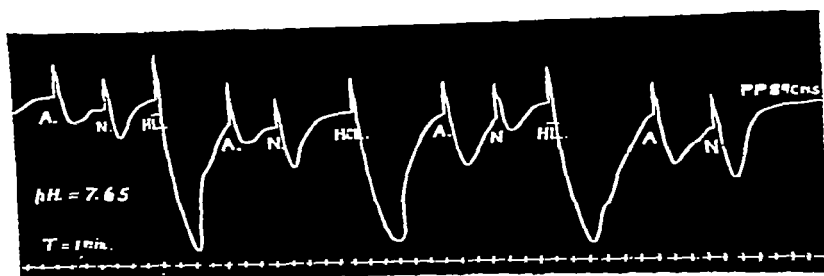
Fig 2 The adjuvant action of lactic acid on the vaso-dilator action of sodium nitrite. The action of the nitrite, as compared with that of the acetyl-choline, is seen to be greater after the injection of lactic acid than after that of an equivalent dose of hydrochloric acid. HL = 0·5 c.c. 1 p c. lactic acid injected. HCL = 0·5 c.c. 0·005 p.c. hydrochloric acid injected. N = 0·5 c.c. 1 p.c. sodium nitrite injected. A = 0·5 c.c. 0·01 p c. acetyl-choline injected. Perfusion pressure = 89 cms. Ringer pH of perfusing fluid = 7 65 Time tracing in minutes.

It is apparent from these experiments that, in a fresh or an alkaline preparation perfused with fluid buffered at pH 7 6–7 7, the hydrogen ion exerts a definite "unlocking effect" on the vaso-dilator actions of acetyl choline and sodium nitrite analogous to that of lactic acid on the capillary dilator action of histamine   It is further apparent that the lactate ion exerts a specific adjuvant effect on the vaso-dilator action of sodium nitrite That the reverse is not true, namely that the chlorine ion exerts a similar effect on the action of choline, is seen from the fact that Ringer's solution contains a large quantity of chlorine ions

These results receive corroboration from, and at the same time afford an explanation for, the otherwise anomalous findings of a large number of experiments

(a) When the vaso-dilator actions of sodium nitrite and acetyl choline had disappeared during perfusion with a fluid of pH 7 3–7 4 it was frequently found that the injection of 1–2 c c of saturated sodium bicarbonate or of dilute caustic soda did not bring about their return If, however, lactic acid was injected after the alkali the action of the two drugs always returned

It would seem then that the "unlocking effect' of the hydrogen ion is dependent on the temporary relaxation of vessels which have been

constricted by alkali That it is not due to any permanent change in the tone of the vessels is seen from the fact that it takes place after the perfusion pressure has returned to its original level, as shown in Figs 1 and 2 It is possible that the effect is due to the temporary relaxation of the larger vessels which have been constricted by the alkali and the consequent flushing through of the peripheral vessels with more alkaline fluid which thus increases their tone

(*b*) On perfusing a fresh or a prepared alkaline animal with fluid buffered at pH 7 3–7 4 there was a gradual fall in the perfusion pressure, accompanied by an increased outflow and the disappearance of the actions of sodium nitrite and acetyl choline Further, if the doses of these drugs were adjusted so that the acetyl choline initially gave a fall in pressure equal to or greater than that of the nitrite, it was found that the effect of the choline decreased much more rapidly than, and finally disappeared before, that of the nitrite

The fluid leaving the vena cava under such conditions was found to have a pH of 6 9–7 0

(*c*) On perfusing a fresh or prepared animal with fluid buffered at pH 7 6, and then changing to one buffered at pH 7 0, there was a rapid fall in perfusion pressure, accompanied by an increased outflow and the disappearance of the actions of nitrite and acetyl choline But in this case, although the doses were such that the fall in pressure caused by the nitrite was initially greater than that caused by the choline, yet the nitrite effect decreased more rapidly than, and disappeared before, that of the acetyl choline

The fluid leaving the vena cava under such conditions was found to have a pH of 7 0–7 2

On changing back at once to fluid buffered at pH 7 6 the actions of the drugs returned in the order acetyl choline, nitrite

These results were obtained many times, the fluids in all cases having the same temperature and composition (except as regards their hydrogen ion content)

There was only one difference between the conditions of the experiments (*b*) and (*c*) to account for the diametrically opposite results

In (*b*) the perfusing fluid was buffered at about functional neutrality (pH 7 4) so that the gradual loss of vessel tone must be due to the accumulation of lactic acid in the tissues This is further seen by the fact that the fluid leaving the tissues was more acid than that entering them

In (*c*) the more rapid loss of tone must be due to the direct effect of the functionally acid perfusing fluid This is further seen by the fact that

the fluid leaving the tissues was usually more alkaline than that entering them.

Now it is in (b) that the nitrite effect remains longer than that of acetyl choline, and it is precisely this experiment in which the acid (shown by Hemingway and McDowall to be lactic acid) accumulates, while in (c) the tone is lost before the lactic acid can accumulate and the choline effect lasts longer than that of nitrite

It is clear, then, that the nitrite requires a greater degree of alkalinity and tone of the vessels for the production of its vaso-dilator action than does choline, but that when the loss of alkalinity is accompanied by an accumulation of lactate ions, as in experiment (b), these ions exert their adjuvant effect on the action of the nitrite which thus persists longer than that of the acetyl choline

SUMMARY

(1) A species of "unlocking effect" of the hydrogen ion on the vaso-dilator action of sodium nitrite and acetyl choline is mentioned, and compared with a similar effect of lactic acid on the capillary dilator action of histamine previously described[1]

(2) A specific adjuvant action of the lactate ion on the vaso-dilator action of sodium nitrite is described, and is used to explain certain anomalous results obtained in comparing the vaso-dilator actions of acetyl choline and sodium nitrite in limbs perfused with fluids buffered at varying pH's

I am greatly indebted to Prof McDowall for much kind assistance

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PROTRACTED ŒSTRUS INDUCED BY OVARIAN EXTRACTS   By ROBERT TUISK

(Institute of Physiology, University of Tartu, Estonia[1])

Allen and Doisy[1] induced a prolonged œstrus by continuing to inject liquor folliculi for several consecutive days They made similar experiments with extracts of follicular fluid, the characteristic cornification of the vaginal mucosa continued for $4\frac{1}{2}$ days Frank, Kingery and Gustavson[2] record having maintained a positive vaginal smear in immature rats for from 8 to 9 days, by continuing the injections

The statements of Allen and Doisy are of a great theoretical interest in connection with the problem of the mechanism of the sexual rhythm If it can be demonstrated that the somatic substratum of the œstral phenomena is capable of reacting continuously to a continuous injection of ovarian hormones, then it is clear that the sexual rhythm repeats an ovarian rhythm, if, on the contrary, the somatic substratum reacts discontinuously though ovarian hormones are continuously present, then one must conclude that the sexual rhythm depends upon changes in the sensitiveness of the tissues Brouha and Simonnet[3] repeated the experiments of Allen and Doisy with special reference to the question here discussed According to them, rats injected during 30 days with follicular extracts reveal indeed a prolonged œstrus, but the latter lasts only 4 to 5 days and then a diœstral phase of 1 to 2 days takes place Allen and Doisy[1] also stated that in several experiments in which by repeated injections the œstrus was extended, a leucocytic infiltration was present in the smears But they interpreted this as caused by a "discontinuous action of the hormones due to difficulty in making the injections serve continuously as does the secretion of the normal follicles during the growth phase of the cycle " Further observations by others corroborate the view taken by Allen and Doisy These authorities drew a parallel between their experiments and the condition in which a cystically degenerate follicle maintains the œstrus, probably by continuous secretion of the follicular hormone The cases of "nympho mania" in the cow are well known, similar conditions were observed by

Allen(4) in the mouse, by Courrier(5) in the guinea-pig Finally, Lipschütz(6), in his comparative experiments with ovarian grafts into male and female castrated guinea-pigs, showed that hyperfeminisation of the male can be considered as a protracted œstral condition caused by persistence, in the graft, of mature follicles with a tendency to cystic degeneration Like Allen and Doisy, and contrary to the statements of Brouha and Simonnet, Lipschutz is of the opinion that we shall always find a prolonged œstrus if in any way the threshold concentration of follicular hormone is maintained in the blood

On account of the contradictory statements as related above, experiments with continuous injection of active ovarian extracts were made, with the special object of examining the question whether a periodic leucocytic infiltration necessarily takes place even when follicular hormones are continuously introduced

The experiments were made both on normal and castrated female mice The follicular extract (a mixture of remains of extracts used in former experiments in this laboratory) was given daily subcutaneously diluted in olive oil. Vaginal smears were made twice daily With 0 2 c c œstral changes set in on the third day and full œstrus was observed about 72 hours after the first injection Three different series of experiments with different quantities injected were made

First series 0 2 c c of the extract was given daily during 8 days, two injections at an interval of 12 hours were made every day The results are given in the following table

TABLE I. 0 2 c.c of follicular extract during 8 days

Exp No		Latent period* hours	Beginning of œstrus† days	Duration of œstrus days	Œstrus after last injection days	Interrupted by leucocytes
1 (40)	Castrated	60	3	6¼	>2	0
2 (41)	Castrated	48	2¼	6¼	<2	0
3 (51) (Fig 1)	Normal	?	1½	3 and 5¼	3	+
4 (52)	Normal	?	1	7	1	0

* Latent period ' means the time between first injection and appearance of pro œstrus i e a very pronounced diminution of leucocytes and appearance of reticular cells or the preliminary stages of the horny scales As the vaginal smears were made twice daily, the error is 12 hours As to the respective values in the normal mice see p 185

† First complete disappearance of leucocytes and horny scales only present in the smear

In both castrated mice the œstral phase of cornification without leucocytes lasted almost 7 days In one of these experiments the œstral condition continued for more than 2 days after the last injection, in the second experiment for almost 2 days In both the normal animals

œstrus set m as early as the day after the first mjection Most probably
this short time of latency is to be explamed by the fact that the mjection
of hormones mterfered with the normal œstrus provoked by the ovaries
in situ The œstral condition lasted m one of these experiments 8 days,
m the second 7 days Individual leucocytes were found m the vaginal
smear between the horny scales on the fourth day m the first of these
cases

The first series leaves no doubt that a prolonged œstrus about five
or more times longer than m the normal animal can be mduced by
continuous mjection of ovarian hormones, without there being a
periodicity m leucocytic mfiltration Only m one of four experiments
leucocytes were once observed m the vaginal smear It is of mterest to
discuss this question more m detail, using the diagram as Corner(7)
did m recording the proportionate relations of leucocytes and epithelial
cells m the vaginal smear of the
monkey In Fig 1 the course of Exp 3
is recorded The top of the ordinates
means cornification without any leuco-
cyte being present m the smear, if
single or more leucocytes are present,
this is mdicated by lowering the ordi-
nate In Case 3 (Fig 1) only a few
leucocytes appeared at the end of the
fourth day of cornification The inter-
pretation of this phenomenon is not an
easy one If not mcidental and with-
out any relation to the hormonic con-

28 IV 29 30 1 V 2 3 4 5 6 7 8 9 10
♀   First Inject   Last Inject

Fig 1

Diagram of Exp 3 of Table I The
greater the quantity of leucocytes the
lower the ordinates Two periods of
prolonged œstrus mterrupted by ap-
pearance of a few leucocytes Œstrus
lasting almost 3 days after last m
jection.

dition, one would suppose that the œstrus was mterrupted for a very
short time or on a limited area of the vaginal mucosa, leucocytic
mfiltration beginning owing to the fact that the respective quantity
of hormones was not sufficient to maintam continuously the threshold
concentration of hormones as already supposed by Allen and Doisy
The following experiments are rather m favour of this suggestion

Second series 0 1 c c of the extract was given m two daily mjections
and repeated during 12 days The results are given m Table II

In Case 1 œstrus set m 3 days after the first mjection, but then
again, during several days, single leucocytes were to be found, and only
6 days afterwards an œstral condition free of leucocytes set m for 3 days
In Case 2 œstrus set m 3 days after first mjection and continued for
7 days without mterruption, then again leucocytes appeared The

TABLE II. 0 1 c.c. of follicular extract during 12 days.

Exp. No		Latent period hours	Beginning of heat days	Duration of œstrus days	Œstrus after last injection days	Interrupted by leucocytes
1 (38) (Fig 2)	Castrated	60	3	3 and 1	0	+ + −
2 (39) (Fig 3)	Castrated	60	3	7	0	0 (leucocytes only at the end of the 7th day)
3 (49)	Normal	0	0	> 13	3	0
4 (50)	Normal	?	1	4, 2 and 1	2	+ + +
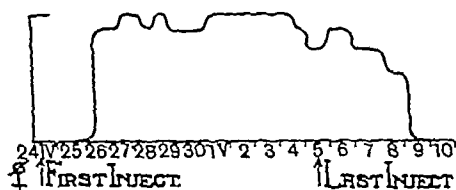
Fig 2

Diagram of Exp 1 of Table II. There are several periods with few leucocytes
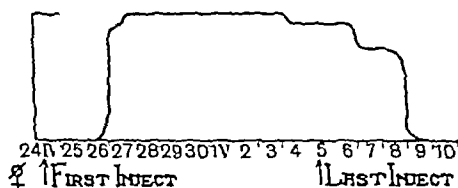
Fig 3

Diagram of Exp 2 of Table II. An uninterrupted œstrus of 7 days Few leucocytes appeared already before last injection.

diagrams (Figs 2 and 3) give a good idea of the condition as observed in these two animals But it must be insisted upon that there never was a regular diœstrus, neither in the cases mentioned here, nor in those of the third series (see below), as long as injections were made In Exp 3, with an uncastrated female, œstrus lasted more than 13 days, 3 of these being after the last injection. In the last experiment (4) œstral phases of 4, 2 and 1 days were interrupted by leucocytes without there being any periodicity as to this

In the second series in which the quantity of hormones daily introduced was smaller than in the first series, only two animals (Cases 2 and 3) revealed long uninterrupted œstrus, whereas both others (Cases 1

and 4) showed shorter œstral phases often interrupted by leucocytes, but, as just mentioned, without any periodicity When compared with the first series it seems very probable that the appearance of the leucocytes in the vaginal smear was caused by temporary lowering of the hormone concentration in the blood This becomes still more evident when discussing our third series of experiments

Third series Three castrated and three normal females were injected during 16 days with about 0 05 c c , once daily, of the same solution as in the first two series

TABLE III. 0 05 c c. of follicular extract during 16 days.

Exp No		Latent period hours	Beginning of heat days	Duration of œstrus days	Œstrus after last injection days	Interrupted by leucocytes
1 (11)	Castrated	60	4	1¼	0	+ + +
2 (12) (Fig 4)	Castrated	60	8	1¼	0	+ + +
3 (13)	Castrated	60	4	5, 1½ and 1½	0	+ + +
4 (31) (Fig 5)	Normal	0	0	See text	—	—
5 (32)	Normal	?	½	6 and 4	0	+ +
6 (33)	Normal	?	½	3½, 1 and 1	1	+ + +
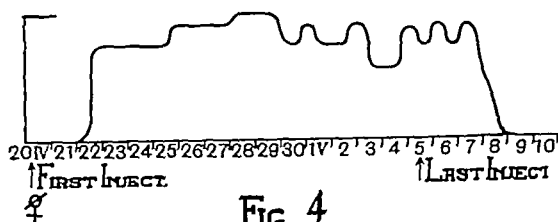
Diagram of Exp 2 of Table III. Full œstrus appearing only about 8 days after first injection. Œstrus lasted only about 1¼ days. Intermediate periods with more or less leucocytes before and after last injection Diœstrus only 3 days after last injection
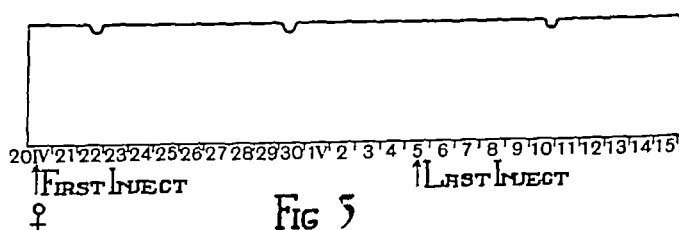
Diagram of Exp 4 of Table III Œstrus before beginning of experiment and lasting 10 days after last injection, *i e.* as long as examined. Twice single leucocytes appeared. Probably a case of "nymphomania "

There was no continuous œstrus in Cases 1 and 2, an œstrus was maintained for 1 day or more in these two experiments In Case 3 œstrus set in 4 days after the first injection and lasted without leucocytes during 5 days Afterwards, days of full œstrus and those with leucocytes followed one another In Case 4, an uncastrated female, there was œstrus already before the experiment began, and this condition lasted not only during the 16 days of injection, but also during 10 days more after the last injection, then the experiment was interrupted During these four weeks single leucocytes were observed three times (Fig 5) It was evidently a case of "nymphomania," as observed by Allen in the mouse In Case 5 the œstrus set in already on the evening of the first day when the first injection was made and this condition lasted for 6 days The leucocytes were present for several days and again for nearly 4 days full œstrus with horny scales only was observed In Case 6 œstrus set in the day after the first injection and lasted 3½ days Later on there were single days with horny scales only

When reviewing the experiments of the third series, we see that there was not a single case without several periods of leucocytic infiltration The quantity injected was sufficient to cause œstrus, as seen especially in Cases 3 and 5 But evidently the quantity was just at the limit of that necessary for maintaining continuously the threshold concentration of hormones in the blood

Two questions ought to be dealt with here The first one is that of the latent period As seen in the tables, the normal mice at the beginning of the experiment were not in diœstrus several were in pro-œstrus (or post-œstrus?), others in œstrus The latent period could not be calculated for these cases, full œstrus set in 12 to 36 hours after the first injection It is not possible to state how far the original ovarian cycle was interfered with here It is, however, conceivable that the injection stimulated the ovaries *in situ* by the intermediation of some unspecific substances, as supposed by Lipschutz[8] in his experiments with ovarian grafts and operative interference on the testicle The influence of similar substances is, according to Lipschutz[9], a question of great practical significance

The second question of interest is that about the condition of the injected animals in the periods of leucocytic infiltration between the periods of full œstrus As already insisted upon, there was never a regular diœstrus without horny scales, either in the castrated animals or in the normal ones, as long as injections were made, and regular diœstrus set in a few days after the last injection These periods were intermediate stages, resembling rather the post-œstrus, horny scales

being present united together in masses   But in general, in the first and second series (large quantities of extract) the number of leucocytes was a minimal one, only a few leucocytes being visible between the horny scales, a condition never seen in dioestrus   As these deviations from the full normal œstrus occurred without any periodicity it seems impossible to consider them as caused by the condition of the somatic substratum, the observations are in favour of the supposition that there were fluctuations in the hormone concentration in the blood, the concentration being sometimes lower than the threshold

SUMMARY

Experiments with continuous injection of extracts of follicular fluid were made in castrated and uncastrated mice

The injections were continued for 8 to 16 days

A continuous œstrus without a single leucocyte in the vaginal smear was maintained in castrated animals as long as 7 days and in uncastrated animals as long as 13 days

In those experiments in which lesser quantities were injected continuously, the œstrus was often interrupted by leucocytic infiltration which, however, was generally not very pronounced, only small numbers of leucocytes being present

The days with leucocytic infiltration occurred without any regularity

It is concluded from these experiments that the mammal reacts with a continuous œstrus when the threshold concentration of follicular hormones is maintained in the body continuously   The failure to obtain continuous œstrus by follicular injection, as in the experiments of Brouha and Simonnet, is to be explained, in accordance with Allen and Doisy, by supposing that in the experiments of the French investigators the threshold concentration of hormones was not maintained

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THE INITIAL PHASE OF REFLEX INHIBITORY RELAXATION IN EXTENSOR MUSCLES

By T GRAHAM BROWN (*Cardiff*)

(From the Physiology Institute, Cardiff)

THE experimental observations described in this paper have a bearing upon the general question Where reflex phenomena vary in intensity with variation in the intensity of the stimulus which evokes them, do the intensities of the reflex phenomena and the intensities of the evoking stimuli show a relationship of simple proportionality?

The classic example of reflex inhibitory relaxation is the inhibition of extensor tone, or of the extensor contraction in the crossed extension-reflex, during the same-sided flexion-reflex In the decerebrate cat, gastrocnemius-soleus muscle contracts in the crossed extension-reflex Sufficiently intense repetitive stimulation of receptor end-organs in the foot of the same side, and applied during this extension reflex, produces inhibitory relaxation of these ankle extensors As far as concerns isotonic records of this phenomenon (mechanical registration), the inhibitory relaxation is not suddenly established at its full extent Where inhibition is evoked by repetitive faradic stimulation (frequency about 20 per sec) continued for several seconds, and where that stimulus is of sub-maximal intensity, the records clearly show that the extensor muscles may still be relaxing (i e their length still increasing) 2 sec , 3 sec , or even longer after the commencement of the inhibitory stimulus

The graphic curve of such a relaxation, if registered upon a surface moving with constant velocity, records the increments of relaxation (muscle lengthening) in consecutive equal intervals of time Were these increments equal, the curve of relaxation would proceed as a straight line sloping down towards the axis of abscissæ—if, as is usual, fall of the writing point registers reflex lengthening (relaxation) of the muscle As a matter of fact the usual curve is more or less sigmoid—approximating to the mirror image of the curve of reflex contraction obtained from the same muscles in the crossed extension-reflex, and the sigmoid character of which has been pointed out by Liddell and Sherrington[1] In that

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The graphic curve of such a relaxation, if registered upon a surface moving with constant velocity, records the increments of relaxation (muscle lengthening) in consecutive equal intervals of time Were these increments equal, the curve of relaxation would proceed as a straight line sloping down towards the axis of abscissæ—if, as is usual, fall of the writing point registers reflex lengthening (relaxation) of the muscle As a matter of fact the usual curve is more or less sigmoid—approximating to the mirror image of the curve of reflex contraction obtained from the same muscles in the crossed extension-reflex, and the sigmoid character of which has been pointed out by Liddell and Sherrington(1) In that

same paper these observers (using the isometric method with optical registration) have shown that the "speed of decline of the muscle tension in inhibitory relaxation is of the same order as that of the relaxation of the mn Tetanus on cessation of the faradisation of the motor nerve itself " They were investigating the vasto-crureus—a knee extensor, and were using maximal inhibitory stimuli Their figures show nearly complete inhibitory relaxation, more or less complete establish ment of which appears to occur about one-fifth of a second after com mencement of the inhibiting stimulus In a later paper these same observers (2) examined the inhibitory effects of stimuli of graded intensity —again using the isometric method with optical registration They find that inhibitory relaxation of tension proceeds more slowly the smaller the intensity of the inhibiting stimulus is Their figures show gradients of relaxation of different steepness, but all approximately rectilinear In other words, the curves of decrease of tension in inhibition make different angles with the axis of abscissae, but are approximately of the same type

In the observations of Liddell and Sherrington, what is registered is the variation from instant to instant of the tension developed in a muscle the length of which is kept approximately constant In my own observations—registration by the isotonic method—what is registered is the change in length of a muscle from instant to instant under approxi mately equal tension Using the latter method, the curve of relaxation in inhibition is usually sigmoid—as has been said above In its first phase (sometimes brief even with stimuli of low intensity, extremely brief when the stimulus is of high intensity) the curve is convex upwards, it then becomes concave and this form is retained (in regular reflexes) for the remaining duration of stimulation Occasionally the first (convex) phase may be as long as 2 sec in duration, where the inhibiting stimulus is of low intensity More usually this phase is briefer Where the inhibiting stimulus is of high intensity the convex phase may become extremely brief—so that the curve in inhibitory relaxation appears to commence suddenly with a perpendicular drop almost at a right angle to the line of preceding contraction, and the sigmoid form almost or completely disappears Where the complete sigmoid form of curve occurs, the record demonstrates that the increments of reflex relaxation in consecutive equal time intervals progressively increase to a maximum and thereafter progressively decrease again The following is an instance (taken almost at haphazard) from my measured curves (Table I), the figures show the fall of a graphic curve in each consecutive 0 25 sec in the period of a

reflex inhibition of gastrocnemius-soleus in the decerebrate cat (The curve, as registered, was measured to 0 25 mm and the figures give a fourfold magnification of actual muscle lengthening)

TABLE I.

	Period of inhibition	Relaxation in mm. (× 4)
1st	0·25 sec.	2 0
2nd	,,	6 0
3rd	,,	3 0
4th	,	1 75
5th	,,	0 75
6th	,,	0 75
7th	,,	0·25
8th	,,	0 25
9th	,,	0 0

Such comparative slowness in the more or less full establishment of inhibitory relaxation might be due to instrumental error in the method of registration adopted (but the recording point of the lever which I use falls through 32 mm in 0 1 sec when the thread attaching the lever to gastrocnemius is suddenly cut) Or it might be due to inherent slowness of relaxation in the muscles used—Fulton and Liddell(3), for instance, state that the rate of relaxation of soleus in the cat is as much as four or five times slower than that of white muscle, and soleus is a component of the ankle extensor which I have been recording Or the slowness might be due to the nature of the spinal centre—so that the discharge of the extensor "half-centre" does not suddenly cease in any reflex inhibition The following experimental observation is inconsistent with all of these possibilities

Present observations The writer(4) has described an increase of inhibitory relaxation as occasionally occurring immediately after the cessation of an inhibitory stimulus

More recently, Liddell and Sherrington(5) have shown that, using the isometric method, there is a further increase in the inhibition of the stretch-reflex in extensor muscles after cessation of the inhibiting stimulus

This terminal increase of inhibitory relaxation ("rebound relaxation after inhibition"—as I originally called it) is naturally best demonstrated where a preceding inhibitory relaxation has been least complete It is sometimes accompanied by a well-marked increase in contraction of antagonistic muscles (e g tibialis anticus) In a recent experiment a well marked example of this phenomenon occurred about seven hours and fifteen minutes after decerebration

Black female cat, 1730 grm., May 14th, 1926

Preparation started 10 35 a.m.   Decerebration performed 11 15 a.m. at end of all operative procedure   Chemical narcosis had been stopped just before decerebration.

The cat was prepared for graphic isotonic registration of tibialis anticus and gastrocnemius soleus of both right and left sides of the body   Remaining muscles of both hind limbs were paralysed by tendon or motor nerve section.   Cat fixed in position for recording about mid day   Needle electrodes inserted under skin of both hind paws for reflex stimulation.   The sacrum, femora, and tibiæ firmly clamped in such a posture that the spinal axis was parallel to the floor with the dorsum of the cat up   The fore part of the cat swung loosely from the ceiling with the fore paws clear of the table   The front part of the cat could therefore take up any posture or perform movements freely
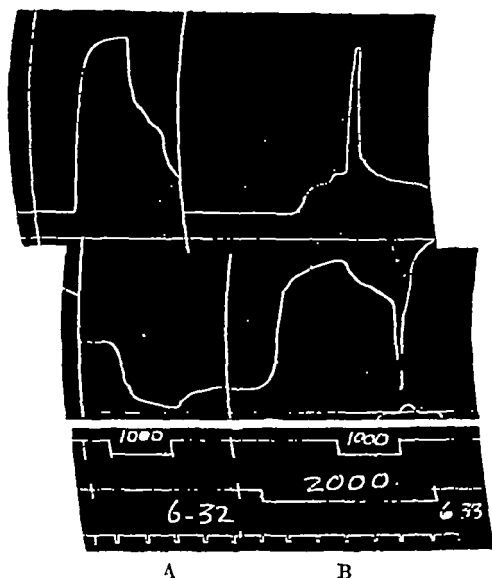
Fig 1   Two reflexes (Nos 17038, 17039, obtained 7 hr 17 min. after decerebration. The first reflex (marked *A*) is a simple flexion reflex   It is not described in the text   In the second reflex (marked *B*) this simple flexion reflex is applied during a "back ground" formed by the crossed extension reflex   It is described in the text

In this figure   Top myograph line = isotonic reflex movement of left tibialis anticus × 2[1], lower myograph line = isotonic reflex movement of left gastrocnemius soleus × 4 —in either case an arbitrary base line is drawn underneath the myogram during the experiment   Upper signal line = commencement (fall of signal) and termination (rise of signal) of repetitive faradic stimulation of left hind paw   Lower signal = the same for right hind paw   Lowest line = time in seconds

NOTE   The irregular line which complicates the gastrocnemius base line in the compound reflex is part of another reflex curve which was taken beneath this one but is not reproduced because it has no bearing upon the present observations

[1] *I c* in the original tracing   It has been reduced to two thirds in the above reproduction.

Results The following instance only need be described

Fig 1   The initial tone of left gastrocnemius-soleus (× 4) was 6 0 mm   A simple crossed extension-reflex was then evoked by repetitive faradic stimulation of the crossed foot   Three sec after the commencement of this stimulation, left gastrocnemius-soleus had shortened by a further 23 75 mm —and would probably have shortened by an additional 0 5–0 75 mm in the ensuing 2 sec had this stimulus remained uninterrupted   But a flexion-producing stimulus (inhibitory as regards this extensor muscle) was super-added at the end of the third 1 0 sec of the extension-reflex and was taken off again at the end of the fifth 1 0 sec   During the 2 sec of the period of this repetitive inhibitory stimulus, the extensor lengthened (relaxed) by about 6 25 mm —so that, at the end of that period, the shortening of the muscle remained 17 5 mm greater than the initial tone   The inhibitory lengthening (relaxation) of the muscle when the inhibiting stimulus was stopped was about 21 p c of the reflex shortening (reflex + tone) which it reduced   For a brief latency (of about 0 2 sec) this inhibitory lengthening continued at the same rate as that during the latter part of actual inhibition (about 4 mm per sec) Then a very sudden lengthening of the muscle occurred   This was such that the curve fell through 18 5 mm in about 0 23 sec   This fall of the lever was immediately followed by as rapid a rise, and the double movement was accompanied by a reciprocal contraction and relaxation in the flexor (tibialis anticus)   At the lowest point in the curve of the "terminal" extensor relaxation, the muscle was still a few mm shorter than its post-mortem length

This instance of reflex phenomena registered by the isotonic method is given because it shows the comparative "dead-beatness" of the method for reflex relaxation   It demonstrates in one and the same reflex record widely differing rates of inhibitory relaxation, and thus confirms with the isotonic method the observation of Liddell and Sherrington that, with the isometric method, the rate of establishment of inhibition may vary

In this instance we have In the *first* place, a comparatively slow and incomplete extensor relaxation occurring during the period of the inhibitory stimulus   In the *second* place, a very rapid and nearly complete relaxation (about 20 times as fast as the first) occurring immediately after the termination of the inhibiting stimulus   A point of importance is that the present experiment exhibits these two widely different rates of inhibitory relaxation *in one and the same reflex*—that is, under the same general conditions (body temperature, blood circulation, respiration, etc)

The rate of the rapid relaxation—18 mm in 0 23 sec —is slower than that of the mechanical fall of the lever when suddenly disconnected from the muscle (32 mm in 0 1 sec) and one out of many possibilities is that the difference in those two rates of fall may be due to the mechanical resistance of the shortened muscle to the distortion of its own shape during lengthening But this comparatively small difference in speed of fall is of a quite different order to the difference in speed of fall between the relaxation during inhibitory stimulation and the terminal relaxation here illustrated

The inference seems to be that in certain circumstances the discharge of the extensor "half-centre" is capable of ceasing with great suddenness —perhaps instantaneously, but that the cessation of discharge during the inhibition which is part of the same-sided flexion-reflex is normally a much more gradual process The initial phase of inhibitory relaxation in the flexion-reflex—the phase which elapses before more or less com plete establishment of inhibitory relaxation—is a comparatively long drawn out one, whether the inhibition acts against extensor tone only or acts against the crossed extension-reflex, but in certain reflex condi tions the inhibitory relaxation may be established with great suddenness The prolongation of the initial phase of extensor inhibitory relaxation in the flexion-reflex is therefore a peculiarity of the *reflex* and not an inherent property of the centre

My own results therefore confirm those of Liddell and Sherrington that the rate of inhibitory relaxation of extensor muscles in the flexion reflex may exhibit wide degrees of variation, but they extend the obser vation by showing that the variation is not due to any change in the general condition of the preparation I have also found that in the case of intense inhibitory stimulation the speed of relaxation does not markedly differ from the speed of relaxation in the "*terminal*" relaxation which I have figured in this paper In other words, if intense inhibiting stimuli are used, the rate of relaxation approximates to that in the nerve muscle preparation, and in the case of stimuli which evoke inhibitory relaxation of small magnitude this approximation does not obtain

The question, which is the main object of this paper, may now be approached Is the shape (type) of the curve of inhibitory relaxation the same where the rates of relaxation differ?

The shape of the curve of inhibitory relaxation gives the proportional rate of establishment of the final effect Suppose that the process of establishment was such that equal increments of relaxation occurred in equal periods of time, the shape of the curve would be rectilinear But

if the ultimate maxima varied in different cases (although occurring at the same instant in the period of inhibition), the rectilinear curves would make different angles with the axis of abscissæ. In other words, the rates of relaxation would vary as between different curves, but the type of all curves—namely, rectilinear—would be the same. Should similarity of type be established experimentally, a relationship of simple proportionality between the inhibitory relaxations (at any given instant) and the intensity of the stimuli which evoke them would be shown—for the above argument applies also to types of curve other than rectilinear

Liddell and Sherrington(6), contrasting the general courses of strong and of weak inhibitory relaxations, note that the terminal 50 p c of relaxation may be only of twice the duration of the initial 50 p c in the weaker relaxation—whereas it may be several times the duration in the stronger, and they also note the more rounded initial part of the curve in the first part of the inhibitory fall. In maximal inhibition, the cutting off of discharge in the motoneurones appears to be simultaneous, but, in the case of sub-maximal inhibition, they note that "inhibitory relaxation of such character [as that described above] indicates that the arrest of the discharging activity of the motoneurones is in the earlier period of the relaxation only partial, although by successive increase it finally becomes 'total,' i e ultimately extinguishes discharge from all the motoneurones engaged in emitting motor impulses to the muscle". They do not further investigate the question of the variation in shape of the curve of inhibitory relaxation

In order to illustrate the effects of intensity of stimulus upon the shape of the curve of inhibitory relaxation (lengthening), figures from the latest series of reflexes which I have measured for another purpose may be quoted (Table 2). In each case the inhibition acts against an initial extensor tone, and not against the artificially evoked extensor-reflex. Save in the case of observation 7 (extensor tone 17 0 mm), the initial tone is comparatively steady—the maximum (observation 3) being 28 75 mm, and the minimum (observation 9) being 21 0 mm. In this experiment the duration of the inhibiting stimulus was 2 sec, and its intensity was progressively raised in consecutive reflexes. At low intensities, the curve of inhibitory relaxation was convex upwards—fall of the curve towards the axis of abscissæ denoting increase in relaxation (length) of the muscle. An intermediate intensity of stimulation evoked a relaxation the curve of which was sigmoid during the 2 sec of its duration—at first convex upwards and then concave. With strong

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by the ratios in column vi Where that ratio is less than unity, the rate of relaxation is greater in the second 1 0 sec of stimulation, unity itself would mean equal rates in the first and second 1 0 sec of inhibition, and values greater than unity denote a greater rate in the first 1 0 sec of stimulation As a matter of fact, in the later observations almost the whole of the inhibitory relaxation occurs during the first 1 0 sec of inhibition, and comparatively little relaxation occurs in the second 1 0 sec —the relaxation has become almost maximal during the first 1 0 sec of stimulation

This then demonstrates the fact, seen in almost all well-graded isotonic series of inhibitory relaxations, that both the gradient and shape of the curve of relaxation vary with the intensity of the inhibiting stimulus The inference is that the inhibitory relaxation at any given instant in the period of inhibition does not bear a relation of simple proportionality to the intensity of the stimulus which evokes that relaxation

SUMMARY

1 The discharge of the extensor "half-centre" (mass of efferent neurones supplying extensor muscles which act on the ankle) may cease with great suddenness under certain reflex conditions (e g in the phenomenon of terminal relaxation after sub-maximal inhibition)

2 In flexion-reflexes of sub-maximal intensity, inhibitory relaxation of extensor muscles (as shown by the isotonic graphic method of registration) may be comparatively slowly established

3 These two different rates of inhibitory relaxation may occur in one and the same reflex graph (as here illustrated)

4 The comparative slowness of inhibitory relaxation in the flexion-reflex of the hind limb of the decerebrate cat is therefore not due to an inherent property of the spinal centres It is a property of the flexion-reflex as such

5 Examination of graded series of inhibitory relaxations (of extensor tone) in flexion-reflexes of varying intensity (i e evoked by stimuli of varying intensity) shows that the rate of inhibitory relaxation (muscle lengthening) of the extensor muscles in the flexion-reflex increases with increase in the intensity of the reflex (i e of the stimulus which evokes it)

6 A graded series of inhibitory relaxations (in extensor muscles during the flexion-reflex) evoked by stimuli of varying intensity may give curves the shapes of which vary in type with variation in the

intensities of stimulation the curve became almost purely concave This gradual change in shape of the consecutive curves was brought about by the progressively earlier appearance of the point of maximum rate of relaxation as the intensity of stimulation was increased

This phenomenon can be demonstrated approximately by giving the ratios between the magnitudes of relaxation (lengthening) in the first 1 0 sec and in the second 1 0 sec of the period of the inhibiting stimulus In the following table (Table II) the first column gives the number of the observation in order of increasing intensity of stimulation (this is also the order in temporal sequence), the second column gives the amount of initial tone ($\times 4$) measured in mm of reflex shortening, the third column (marked A) gives the amount of relaxation (reflex lengthening $\times 4$) during the first 1 0 sec of the period of inhibition, the fourth column (marked B) similarly gives the amount of reflex lengthening in the second 1 0 sec of the period of inhibition, the fifth column (marked $A + B$) gives the total relaxation in the 2 sec of inhibition the sixth column gives the ratio $A \quad B$

TABLE II

i	ii	iii	iv	v	vi
			Inhibitory relaxation		
				$(A + B)$	
No of	Initial	A	B	Total	
observa	tone	0 0–1 0 sec	1 0–2 0 sec	0 0–2 0 sec	Ratio
tion	mm ($\times 4$)	mm ($\times 4$)	mm ($\times 4$)	mm ($\times 4$)	($A \quad B$)
1	27 5	0 0	1 0	1 0	0 0
2	27 25	0 5	2 0	2 5	0 25
3	28 75	1 5	5 75	7 25	0 26
4	28 5	4 25	12 25	16 5	0 35
5	27 75	5 5	14 25	19 75	0 39
6	26 0	7 5	11 5	19 0	0 65
7	17 0	11 0	2 5	13 5	4 40
8	23 0	16 5	1 5	18 0	11 00
9	21 0	16 25	1 5	17 75	10 83

It will be observed that the amount of relaxation in the first 1 0 sec of stimulation increases throughout the series with increasing intensity of stimulation, the amount of relaxation in the second 1 0 sec increases up to observation No 5, and thereafter decreases again, the total amount of relaxation in the whole period of 2 sec inhibition increases up to observation No 5, and thereafter remains comparatively stationary In the earlier observations, the major part of the reflex relaxation there fore occurs in the latter part of the period of inhibition, whereas in the later observations the major part of the inhibitory relaxation occurs in the earlier part of the period of inhibition This change is demonstrated

UPON INHIBITORY RELAXATIONS EVOKED BY REFLEX STIMULI OF CONSTANT INTENSITY ACTING AGAINST VARIED MAGNITUDES OF EXTENSOR TONE

By T GRAHAM BROWN (*Cardiff*)

(From the Physiology Institute Cardiff)

A SUBMAXIMAL flexion-producing stimulus (repetitive faradic shocks with a frequency of 20 per sec continued for a few seconds of time) evokes inhibitory relaxation of the tone of ankle extensors of the hind limb to which the stimulus is applied in a decerebrate cat If a crossed extension-reflex is in being at the time when this flexion-producing stimulus is applied, the stimulus evokes a similar inhibitory relaxation of the reflex contraction of the extensors Inspection of a large number of graphic records of isotonic curves of such reactions clearly demonstrates difference (at any given instant in the period of inhibition) in absolute magnitude between the inhibitory relaxation of extensor tone and that of reflex extensor contraction when these two "backgrounds" of contraction are acted upon by inhibitory stimuli of the same intensity Sometimes the ' reflex ' relaxation is notably smaller[1] in magnitude than the "tonic" more often the reverse is the case

The writer suspected that measurement of the inhibitory relaxations in a series of constant flexion-reflexes, where each acts against a different magnitude of extensor tone might demonstrate a lack of simple proportional relationship between the magnitude of initial extensor tone and the magnitude of the inhibitory relaxation to which it is subjected. In order to investigate this point, measurements which had previously been made of some reflex series for another purpose were examined.

If a series of crossed extension-reflexes of increasing magnitude is evoked by a series of reflex stimuli each of which is of greater intensity than its immediate predecessor and if the stimulations of the series last each for 2 0 sec (*e g*) and are applied at regular intervals of 2 min the residual extensor tone which is in being at 1 min (*e g*) after each reflex of the series often shows increase in magnitude as the series progresses

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[1] A figure published in a previous paper [Graham Brown, The Journ. 63. p 187, 1927 (Fig 1, on p 190)] demonstrates this.

intensity of stimulation That is, in these cases the inhibitory relaxation at any given instant in the period of inhibition does not bear a relation of simple proportionality to the intensity of the stimulus which evokes that relaxation

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The writer suspected that measurement of the inhibitory relaxations in a series of constant flexion-reflexes, where each acts against a different magnitude of extensor tone, might demonstrate a lack of simple proportional relationship between the magnitude of initial extensor tone and the magnitude of the inhibitory relaxation to which it is subjected In order to investigate this point, measurements which had previously been made of some reflex series for another purpose were examined

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[1] A figure published in a previous paper [Graham Brown, This Journ. 63 p 187, 1927 (Fig 1, on p 190)] demonstrates this

An initial tone of less than 4 mm (this figure representing true shortening of the muscle × 4) at the commencement of the series of extension reflexes may rise to a tone of more than 27 mm after the 6th member of the series If, now, flexion-producing stimuli of constant intensity (repetitive faradic shocks continuing in each case for 2 sec) are applied mid-way in the intervals between the ascending series of extension reflexes, the inhibitory effects produced by them will occur against increasing "back-grounds" of extensor tone

Comparatively great variation in initial extensor tone, and the use of a clearly sub-maximal inhibition to produce the relaxations, are conditions for the present experiment When these conditions obtain, a simple manner of demonstrating the presence or absence of a relation ship of simple proportionality between the magnitude of inhibitory relaxation and the magnitude of the extensor tone which is relaxed is given by calculation of the ratio—relaxation initial tone

The magnitudes of the initial tone and of inhibitory relaxation are obtainable from isotonic records by measuring the heights of points in the reflex curve above an arbitrary base line which is drawn mechanically during the registration of the reflex From these magnitudes a constant has to be subtracted—this constant is the similar height (above the arbitrary base line) of the curve when the extensor is in its maximal relaxation

The following table (Table I) gives the results of such an experiment The first column gives the number of the observation in temporal sequence, the second column (*A*) gives the magnitude of initial extensor tone which obtained just before[1] the commencement of a constant in hibitory stimulus, the third column (*B*) gives the amount of relaxation of this tone which had occurred 2 sec after the commencement of the

TABLE I

i	ii	iii	iv
		Relaxation at	
	Initial tone	2 0 sec	
Order in	(*A*)	(*B*)	
time	mm (×4)	mm (×4)	(*B A*)
1	3 75	2 0	0 53
2	6 75	2 75	0 41
3	9 0	4 5	0 50
4	18 5	9 75	0 53
5	21 5	14 25	0 66
6	23 5	9 75	0 41
7	27 5	15 75	0 57

[1] The tone had been maintained steadily at the given magnitude for some time before the commencement of inhibition

inhibitory stimulus, the fourth column (B A) gives the ratio obtained by dividing the relaxation by the initial tone

In this experiment, which was obtained in a decerebrate cat about 4 hr after decerebration, it will be observed that the initial tone rises in the same order as the temporal sequence of the inhibiting reflexes In another similar experiment, obtained in the same cat about 40 min later, the tone was more variable in relation to temporal sequence (Table II)—thus excluding, to a certain extent, the possibility of "fatigue" factors from complicating the results

The description for Table I applies equally to Table II

TABLE II.

I	II	III	IV
		Relaxation at 2 0 sec.	
Order in time	Initial tone (A) mm. ($\times 4$)	(B) mm ($\times 4$)	(B A)
1	3 75	1 75	0 47
9	6 5	1 5	0 23
2	8 5	1 75	0 21
10	10 75	2 0	0 19
8	16 0	3 25	0 20
3	18 0	3 0	0 17
5	21 0	4 5	0 21
4	25 0	5 25	0 21
6	25·75	5 0	0 19
7	28 25	6 5	0 23

The comparative constancy of the ratios given in the fourth columns of these two tables (excepting the first ratio in Table II) is somewhat remarkable in view of the possible sources of error Constancy of this ratio means that the inhibitory relaxation bears a relation of simple proportionality to the magnitude of tone which is subjected to the inhibitory relaxation Reflex stimuli of the same intensities acting against differing magnitudes of extensor tone might conceivably always give the same magnitude of inhibitory relaxation, or conceivably they might always relax (lengthen) the muscle until the same absolute magnitude of residual contraction remained in each case, or inhibitory relaxation might vary in magnitude with variation in the magnitude of the initial tone The measurements here given show that, at any rate at this time in this particular experiment, the third of these three possibilities obtained The inhibitory relaxation varies here proportionally with the initial tone, and the relationship is approximately one of simple proportionality stimuli of constant intensity here cause inhibitory relaxations of approximately equal *proportions* of the initial tone

This experiment is described here largely because, in itself compara tively convincing, the result is not what was anticipated It must how ever be taken without prejudice to the observation that a relationship of simple proportionality does not necessarily obtain between the in hibitory relaxations evoked by the same stimulus against initial tone alone on the one hand, and against extensor reflex contraction on the other hand Nevertheless the occurrence here of approximately this special relationship of simple proportionality is a striking one To argue on the basis of a similar relationship of simple proportionality between the magnitude of reflex shortening or lengthening and the magnitude of the increase of discharge ("excitation") or decrease of discharge ("in hibition") in the spinal centre would be rash But it may be noted that a relationship of simple proportionality between excitation and inhibition will obtain in the centre if the nerve impulses which condition inhibition are conveyed in a haphazard manner to discharging and non-discharging efferent neurones But again there are, no doubt, many other possible functional arrangements in which this relationship will also obtain

SUMMARY

1 An experiment is described in which the inhibitions evoked in a series of constant sub-maximal flexion-reflexes act against initial extensor tones of different magnitudes The inhibitory relaxations thus produced are of greater magnitude the greater is the initial tone But treatment of the measurements obtained in this experiment shows that the relaxations in each instance are approximately the same ratio of the initial tones which are relaxed

2 In this experiment there is an approximately close relationship of simple proportionality between the magnitude of inhibitory relaxation and the magnitude of preceding initial extensor tone

THE DEPRESSOR (VASO-DILATOR) ACTION
OF ADRENALINE

By H H DALE AND A. N RICHARDS (*Philadelphia*)

(From the National Institute for Medical Research, Hampstead)

INTRODUCTORY

IN a recent paper, mainly concerned with the action of histamine, Burn
and Dale(1) described some experiments on the vaso-dilator action
produced in Carnivora by small intravenous injections of adrenaline
They observed an apparent contrast between the effects of injections so
given, and injections of equal amounts of adrenaline made centrally into
the left subclavian artery The vaso-dilator effect following the venous
was regularly greater, and quicker in onset by some 6 sec , than that
following the arterial injection They interpreted this difference as an
indication that the vaso-dilator effect was not due to the direct action
of adrenaline, but to that of a histamine-like principle liberated from the
lung as the adrenaline passed through its vessels This conclusion was
the more attractive, in that it appeared to throw light on the difficulty,
which we had indicated some years ago(2), in reconciling this vaso-
dilator action of small doses of adrenaline, or of larger doses very slowly
injected, with the evidence suggesting that adrenaline is a factor in the
maintenance of vascular tone, especially in vessels which have regained
their tone after denervation Burn and Dale's suggestion seemed to
gain further interest from the recently published observation by Best,
Dale, Dudley and Thorpe, that the lung is distinguished among the
organs of the body by the high yield of histamine obtainable from it, by
simple alcoholic extraction It was in the hope of obtaining further
evidence concerning the origin of this histamine in the lung, and on the
mechanism of its supposed liberation in response to adrenaline, that the
experiments here described were undertaken It will be seen that their
main outcome has been to show that Burn and Dale's results were due
to a technical error, and to remove any ground for the suggestion that
the lung has any special function of liberating histamine or any other
vaso-dilator substance into the blood stream, in response to adrenaline

1 *Adrenaline vaso-dilatation in the dog* Burn and Dale had made their arterial injections into the central stump of the subclavian artery, after tying off, as they supposed, all the branches between the cannula and the origin of the artery from the aortic arch We shall return to this point later At the outset of our experiments we wished to exclude the possibility that part of the injection might be left in the subclavian artery, and that a significant reduction of the quantity reaching the aortic stream immediately might thus be produced With the object of avoiding this, and of introducing undoubtedly equal doses under like conditions into the blood, before and after its passage through the lungs, we decided to compare the effects of equal injections into the right and left auricles respectively In the cat we found this method impracticable Exposure of the heart in this species, either by laying the chest open under artificial respiration, or by Drinker's(3) method of bringing the heart to the surface, so alters the conditions of the circulation that the depressor, vaso-dilator effects of adrenaline can no longer be demon strated In the dog, on the other hand, the depressor effect is still pro duced, in typical form, with the chest open

The dogs were first anæsthetised with ether, and chloralose was then given intravenously in such a dose that, with a little ether added to the air supplied by artificial respiration, a deep anæsthesia was maintained The chest was opened by splitting the sternum, and injections could then be made by inserting the needle of the syringe alternately through the wall of the right and of the left auricular appendix Fig 1 shows the results of such a pair of injections The fall of blood-pressure resulting from injection into the left side of the heart was practically identical with that caused by injection into the right side, it was certainly not smaller and followed the injection at an interval shorter by 1 or 2 sec There was no sign of the contrast described by Burn and Dale Later we compared the effects of injections made by a method more nearly resembling that used by Burn and Dale The blood of the dog having been rendered incoagulable by an injection of heparin, a cannula made of thick-walled capillary glass tubing (thermometer tubing), big enough to fill the lumen of the left subclavian or carotid artery, and closed at its outer end by means of a clamped rubber tube, was pushed down the central end of the artery, till its open tip rested in the lumen of the aortic arch, or, in some cases, in the upper part of the thoracic aorta The cannula was then tied in position by a thread round the artery near

the point of insertion   It had previously been filled with saline, and its
volume had been determined, so that a corresponding addition to the
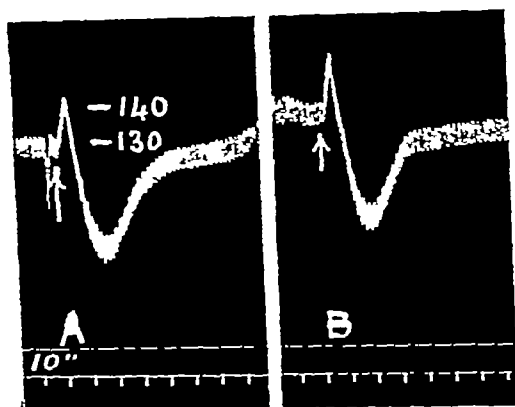
Fig. 1 Dog   Ether   Chloralose
Chest open. Carotid B P   Injection of 0 1 mgm. adrenaline.
At *A* into right auricle at *B* into left auricle.

volume of adrenaline solution delivered into it from a syringe compen-
sated accurately for this dead-space   Equal injections of a 1 in 100,000
solution of adrenaline were then made alternately into the lower end of
the jugular vein, and directly into the aorta through the capillary
cannula   Again there was no significant difference in the depths of the
depressor effects, and again the fall of pressure following the aortic
injection had a latent period shorter by a second or two than that follow-
ing the jugular injection

The results were so completely different from those obtained in the
cat by B u r n and D a l e, that it was obviously necessary to repeat the
experiments in that species with the improved technique

2 *Adrenaline vaso-dilatation in the cat* We prepared and calibrated
capillary cannulæ, similar to those used in the dog, but of sizes suitable
for the cat's arteries, and with them made injections into the aorta of
the heparinised cat, anæsthetised with ether   The venous injections
were made, as before, by a cannula inserted into the external jugular
vein, close to its entry into the superior vena cava   In some experiments
cats were used in which one sciatic nerve had been cut aseptically under
ether (by H H.D) some days previously, the leg so denervated being
enclosed in a plethysmograph connected to a Brodie's bellows, for a
direct record of the vaso-dilator effect Three experiments may be quoted

In the first the arterial capillary cannula was passed down the left subclavian artery, so that its tip lay in the arch of the aorta A dose of 0 003 mgm of adrenaline injected into the jugular vein caused a fall of arterial pressure practically identical with that caused by the same dose injected into the aortic arch (Fig 2) There was no such contrast as B u r n and D a l e observed The latent period, moreover, of the effect following arterial injection was the shorter

Fig 2   Cat. Ether
Injection of 0 003 mgm. adrenaline. At *A* into the jugular vein, at *B* into arch of aorta.

In the second experiment a larger arterial cannula was pushed down the right carotid artery, and its tip guided past the bend of the aortic arch, so that it rested, as post-mortem examination showed, in the thoracic aorta, about 2 cm below the origin of the left subclavian artery The volume of the left hind leg, in which the sciatic nerve had been cut four days previously, was recorded, as well as the pressure in the left carotid artery Fig 3 shows the effects produced by a venous and an arterial injection, each of 0 0032 mgm adrenaline The fall of arterial pressure following the arterial injection is, in this case, distinctly larger and has a shorter latency than that following the venous injection The effects on the volume of the denervated leg show no definite differences

In the third experiment we did not aim at comparing the effects on the general arterial pressure following the two forms of injection Our main object was to study the dimensions and promptitude of the vasodilator response of a denervated hind leg, to injections made, on the one hand, into the general circulation through the jugular vein, and, on the other, into the arterial stream just before it reached the leg A suitable capillary cannula was, therefore, pushed up the right iliac artery, so that its tip rested between the origin of the inferior mesenteric artery and the bifurcation of the aorta The denervated left leg was enclosed in a plethysmograph for a volume record, and the arterial pressure recorded from the carotid artery Fig 4 shows the results It will be seen that the injection of 0 0004 mgm of adrenaline into the aorta just above its bifurcation, so that a large part of the dose was carried straight
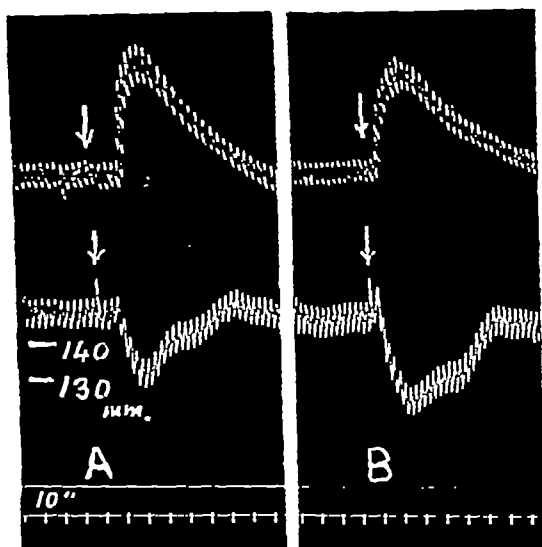
Fig 3   Cat   Ether
Volume of denervated leg and carotid B P   Injection of 0 0032 mgm
adrenaline.   At *A* into jugular vein, at *B* into arch of aorta.
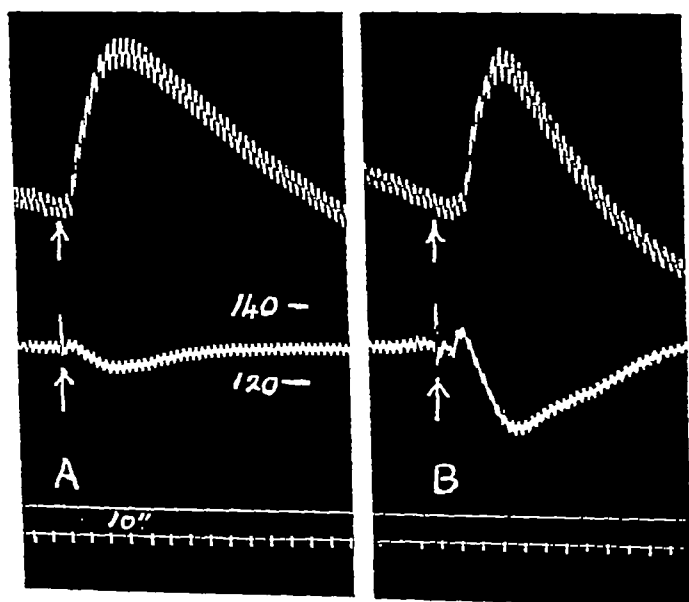
Fig 4. Cat. Ether
Cannula passed up right iliac artery into aorta. Volume of denervated left leg and
carotid B P   At *A* 0 0004 mgm adrenaline into lower end of aorta, at *B* 0·002 mgm.
adrenaline by jugular vein.

to the vessels of the denervated leg, caused a conspicuously larger expansion of the leg, with a much shorter latent period, than that following the venous injection of a dose five times as large   Naturally the effect on the general blood-pressure was much greater with the venous injection

It is perfectly clear, then, that the vaso-dilator effects which we are considering result from the arrival of adrenaline itself in the arterial blood of the organ exhibiting the effect, they are not secondary to the liberation of a vaso-dilator substance in the lung or in any other organ

3   *The source of the earlier error*   B u r n and D a l e supposed that they had ligatured all the branches of the subclavian artery between the cannula and the aortic origin   A simple post-mortem inspection of the collapsed artery after death seemed to confirm this   In a few experiments we followed their procedure, and observed the relative inefficacy and delay in effect of injections made centrally into the subclavian artery with all accessible arteries tied   Our suspicion was aroused, however, by the discovery that when doses of histamine were injected, much smaller than those which B u r n and D a l e had used to control their technique, a similar contrast between the effects of venous and arterial injections appeared   In such cases we killed the animal and injected the subclavian artery from its aortic origin with a plaster cream   It then became clear that the vertebral artery had its origin so low in the chest that it could not be reached without opening the pleura, it was still patent, and the artery which had been ligatured, and mistaken *in vivo* for the vertebral, owing to the similar direction of its main branch, was the costo-

Fig 5   Injected specimen of left subclavian artery and branches of cat.
Th A   Thyroid axis   *Int M*   Internal mammary artery   *C C A*   Costocervical axis   *V*   Vertebral artery   *S C*   Subclavian trunk.

cervical axis (Fig 5)   The weakening of the effect, by the supposed intra-aortic injection, had clearly been due, therefore, to the diversion of the injection through the vertebral artery to the brain, from which

it returned reduced in quantity, to produce a weakened and delayed effect

4 *An abnormal depressor effect of adrenaline* We are convinced that the effect which we have dealt with above, and which is obviously due to a peripheral vaso-dilator action, is the depressor effect of adrenaline which numerous observers have studied in the cat and the dog In the cat we have not observed a depressor adrenaline action of any other type In the dog, however, we have in two experiments observed a depressor action of an obviously different nature, occurring under special conditions At first we were deceived by its resemblance to the ordinary, peripheral effect, and, since others may encounter it, it seems desirable to mention it In a dog under chloralose anæsthesia, with a little added ether, the chest had been opened We had tied both carotid arteries and had passed a cannula down the right one into the aortic arch Both vagi had been cut Preliminary comparisons of the effects of venous and arterial injections, had given results similar to those described above, though the effects were rather weak A cannula was then tied into the central end of the *right* subclavian artery just beyond the origin of the internal mammary, which was then tied, the vertebral being left open The *left* subclavian artery was then tied, proximally to the origin of its vertebral branch, so that the brain of the dog then received blood only through the still open right vertebral artery The tying of this last ligature was followed by a rapid rise of the arterial (left carotid) pressure to a high level, from which it had subsided but little when the experiment was terminated by killing the animal The only apparent cause for this was the restriction of the blood supply to the brain, acting as a stimulus to the vaso-motor centre Under such conditions a new and very powerful depressor action of adrenaline appeared, quite obscuring what was left of the peripheral vaso-dilator action The place of origin of this effect was made evident by the facts that it was most pronounced, and least complicated by an initial pressor phase, and occurred with the shortest latent period, when the injection was made centrally into the right subclavian artery, so that the small dose of adrenaline was carried directly into the still open right vertebral artery Next in order of effectiveness, and shortness of latency, was injection into the jugular vein near its entry into the superior cava Least effective, and most delayed in its effect, was injection into the aortic arch Fig 6 gives a comparative record of the effects of equal injections by these three routes We have not analysed this effect further Since we have only observed it when the tone of the vaso-motor centre has thus been

raised by restricting its blood supply, an effect of adrenaline on this tone seems the most probable explanation This might be a direct effect of
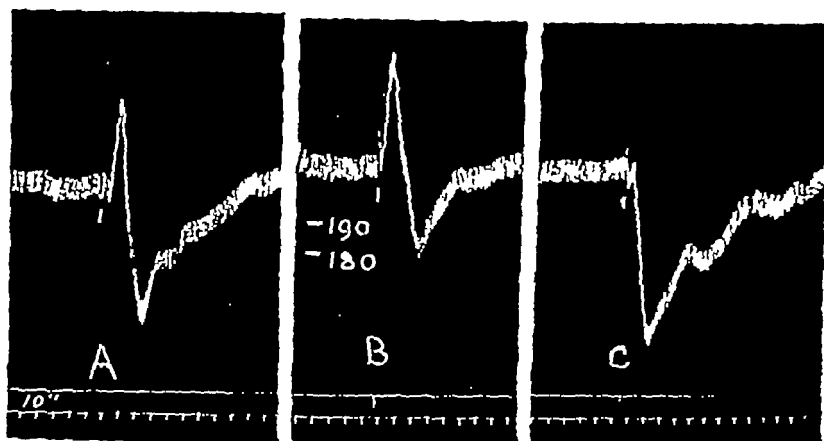
Fig 6 Dog Ether Chloralose

Abnormal depressor effects For description see text. Injections of 0 005 mgm adrenaline. At *A* into jugular vein, at *B* into aorta, at *C* into vertebral artery through right subclavian

adrenaline itself on the nerve-cells, or the secondary effect of an action on the tone of the vessels carrying blood to them If the effect were of the latter kind, the action of adrenaline on the vessels must be a dilator one, that vaso-constriction could not produce it seems to be proved by the fact that clamping the remaining vertebral artery, so as to reduce the blood supply to an anastomotic remnant, had no such effect We have not, however, excluded the possibility of a secondary chemical effect It is just possible that the passage of adrenaline through the intracranial vessels might result in the liberation of a dilator substance, the effect of which only becomes obvious when the general vascular tone has been artificially raised Schafer and Macdonald have already drawn attention to the histamine content of the pituitary body in such a connection Whatever its nature, however, the effect under discussion is an abnormal one, and is not the vaso-dilator action of adrenaline seen under the ordinary conditions of experiment on the anæsthetised dog or cat

5 *Adrenaline vaso-dilatation in artificial perfusion*   One of the considerations which led to the search for an indirect mode of action, to explain the vaso-dilator effects of adrenaline, was the failure to demonstrate this effect on artificially perfused organs, even with the

smallest effective doses   We have recently removed this difficulty also
In two experiments we have perfused the hind limb of a cat with fresh
defibrinated cat's blood, the methods of perfusion, and of recording the
venous outflow and the volume of the limb, being in all details identical
with those used by B u r n and D a l e   In both instances the vessels of the
preparation acquired, early in the experiment, a remarkably high tone,
so that the resistance to perfusion became great, and the perfusion
pressure had to be raised to a high level to secure an adequate flow and
prevent a rapid contraction of the limb volume   The vessels responded
promptly by dilatation to small doses of acetyl-choline and histamine,
injected into the blood stream just before it entered the arterial cannula
Similar injections of very minute doses (0 00005 and 0 0001 mgm) of
adrenaline were then given, and, in both experiments, these regularly
produced a small but quite definite dilator effect, followed by a slower
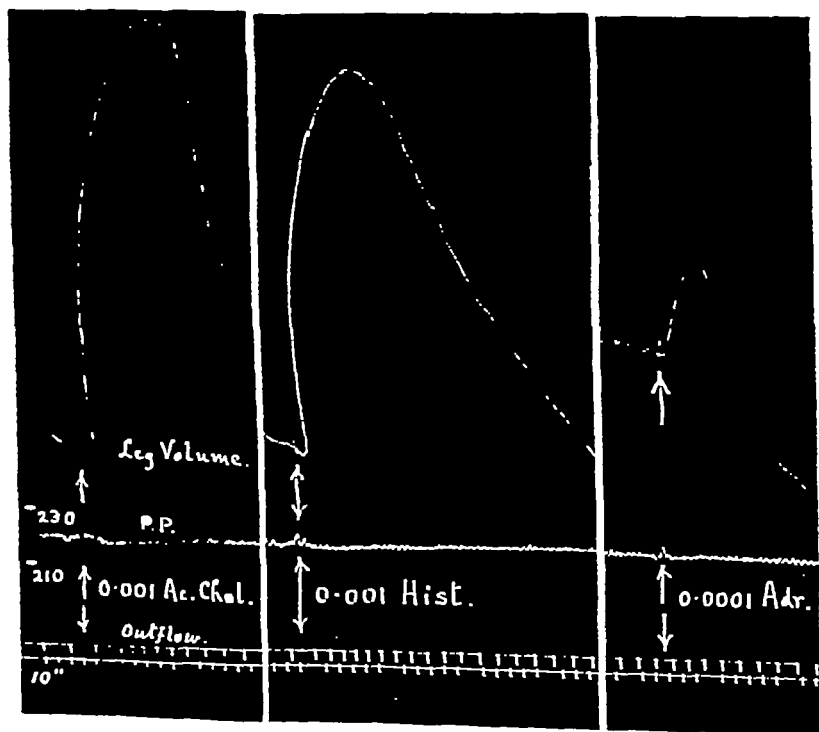
Fig 7   Artificial perfusion of cat's hind limb with defibrinated blood. Record shows, from
above downwards, plethysmogram of limb, perfusion pressure, venous outflow (tilter-
record), time-clock.

constriction The third section of Fig 7 shows such an effect, the effects of 0 001 mgm of acetyl-choline and of histamine being shown for comparison in the first and second sections The dilator effect of the adrenaline on the limb volume is quite definite, and the acceleration of venous outflow corresponding to it is just perceptible, though naturally small It is at least sufficient to exclude the possibility that the expansion might be due to venous constriction

These various experiments make it clear that, whatever the precise mode of its action, the vaso-dilator effect produced by small doses of adrenaline is due to the arrival of that substance itself in the arterial stream, and its entry into the small vessels of the organ in which the reaction occurs, and not to the liberation of vaso-dilator substance in the lung, or in any other organ The question still remains as to the nature of the substance in the blood which maintains, in the denervated minute vessels of the living animal, or in those of the severed limb in such experiments as those just described, a tone which small doses of adrenaline temporarily relax Hitherto it has seemed most reasonable to attribute this tone to adrenaline circulating in the blood, and the question still remains, which we propounded some years ago (1918), whether it is conceivable that the presence of adrenaline, in minute, steady concentration, can maintain a tone which a further, small, sudden injection of the same substance will relax No answer can as yet be given

CONCLUSION

The contrast described by B u r n and D a l e, between the vaso-dilator effects produced by small doses of adrenaline given by venous and arterial injection respectively, was the result of a technical error, and their suggestion that the effect was due to liberation of a histamine like substance from the lung is accordingly not valid The vaso dilator action of adrenaline in the Carnivora, like its vaso-constrictor effects in all species, is due to a peripheral action

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THE ACTION OF HISTAMINE ON THE BLOOD VESSELS OF THE RABBIT

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ONE of the anomalies in the action of histamine has been its failure to produce in rodents, under the ordinary conditions of experiment, the depressor, vaso-dilator action which is an outstanding feature of its action in Carnivora On the arterial blood-pressure of the rabbit, deeply anaesthetised with ether or urethane, it appears to produce very little effect in the small intravenous doses (0 001–0·01 mgm) which cause simple vaso-dilator effects in the cat or dog under similar conditions, in larger doses (0·5–1 0 mgm) it produces a rise of pressure, obviously due to arterial constriction In the exceptional cases when, in the earlier stages of the action of urethane large doses of histamine were observed to cause a sudden fall of arterial pressure in the rabbit, D a l e and L a i d l a w(1) found that this was due to failure of the right side of the heart, as the result of pulmonary obstruction, and the action was accordingly not comparable to the vaso-dilator depression seen in the Carnivora

The point has gained in importance through the suggested relation of the dilator action of histamine on the minute blood vessels, including the capillaries, to the hyperaemia evoked by traumatic stimulation or functional activity of the tissues A large body of evidence in this direction, chiefly from experiments on man, has been produced during recent years, particularly by L e w i s and his co-workers(2) It seems highly improbable that a reaction of such general physiological importance should be produced by an entirely different mechanism in the rodent from that responsible for its appearance in the Carnivora, in man and in other animals According to L e w i s and M a r v i n(3) indeed, a superficial puncture through a drop of dilute (1 10,000) histamine solution, into the skin of the ear of an unanaesthetised rabbit evokes a typical local hyperaemia(3) The failure to observe a depressor effect on the arterial pressure, corresponding to this local dilator action, might be due (1) to a restriction of this type of reaction to certain skin areas, or (2) to such depression of the tone of the capillaries by the anaesthetics

employed, that histamine no longer produced an effective dilatation, but showed only its constrictor effect on the arteries I have accordingly examined the effect of histamine on the arterial pressure of rabbits anæsthetised with chloralose, an anæsthetic which seemed less likely to weaken the physiological tone of the capillaries, or decerebrated under ether, which was subsequently removed by artificial respiration with pure air I have also observed the effect of injections of histamine into the general circulation, without anæsthesia, on the vessels of the completely denervated ear of the rabbit

EXPERIMENTAL

1 *Rabbits under chloralose* The rabbits were anæsthetised by slow injection into an ear vein of a warm, saturated solution of chloralose in physiological saline solution The injection was so regulated as not to produce stoppage of the respiration When 10 c c per kgm had been injected the rabbit was left for about 10 min , and was then found to be deeply anæsthetised The arterial pressure was recorded from a carotid artery and injections made through a cannula tied into the femoral vein, or, in some cases, by a hypodermic needle into the vein of an ear The vagi were cut In rabbits so prepared, with a steady arterial pressure of moderate height (70–100 mm), injections of histamine in quantities from 0 02–0 06 mgm produced, in nearly all cases, a clear fall of arterial pressure Smaller doses (0 01 mgm or less) never produced any definite effect, so that the reaction was very much less sensitive than that seen in the Carnivora In two rabbits only, histamine caused a rise of arterial pressure, followed by a more prolonged fall

The effects have not been completely analysed In most cases a smooth curve of depression, like that shown in Fig 1, was produced In others the injection was followed by a more sudden preliminary fall, which, on the analogy of the effects of larger doses on the cat[1], could be regarded as probably due to pulmonary constriction, this was followed by a small secondary rise, which never carried the pressure back to the original level, and this was followed by the main depressor phase of the effect Fig 2 shows an effect of this more complex type The occurrence of all intermediate forms, between the simple pressor and the simple depressor effect, justifies the assumption that in the rabbit, as in the cat, histamine has two actions, a vaso-constrictor and a vaso-dilator action, operating at different levels in the vascular branching[2]

[1] See Dale and Laidlaw This Journ. 52 p 355 1919
[2] Cf Dale and Richards[4], Burn and Dale[5]

2 *Spinal rabbits* The rabbits were fully anæsthetised with ether, an opening was made in the skull and the whole brain, including the
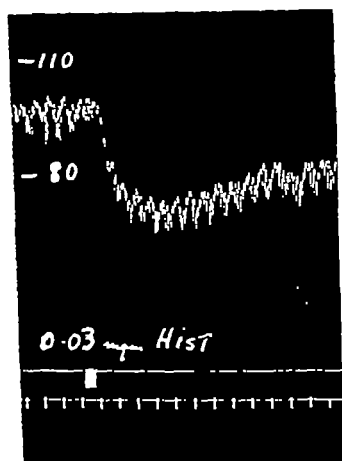
Fig 1 Rabbit Chloralose.
Carotid B P
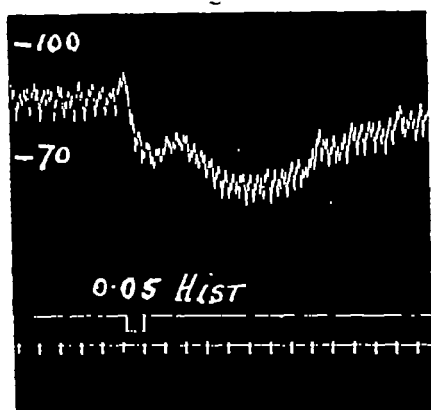
Fig 2 Rabbit. Chloralose Carotid B P

respiratory centre, was destroyed   Artificial respiration with pure air was then applied, and arrangements made for record and injection as in the experiments under chloralose Four such experiments were made The arterial blood-pressure in these spinal animals was naturally low (30–40 mm.) Nevertheless, in two out of the four experiments, injections of histamine produced depressor effects of the same general type as
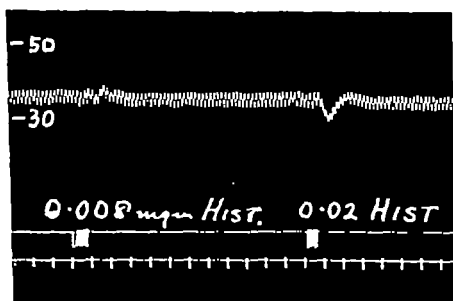
Fig 3   Spinal rabbit. Carotid B P

those seen under chloralose (Figs 3 and 4) In the third rabbit histamine produced a purely pressor response In the fourth rabbit the first in-

jection (0 04 mgm) caused a small fall of pressure followed by a return
to a level above that before the injection, with successive injections the
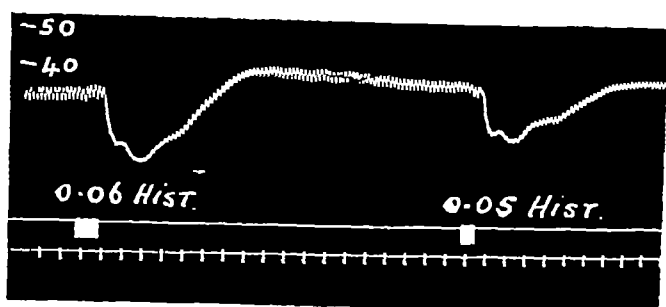
Fig 4. Continuation of Fig 3

depressor phase then became smaller and the pressor phase increased,
until it became predominant

3 *Rabbits under ether* In most cases the effect observed was the
pressor action described by previous observers In one case, however,
in the early stage of the experiment, when the ether anæsthesia was
light though effective, histamine showed a small depressor action, later
in the same experiment, when the anæsthesia had lasted longer and
become deeper, this was replaced by the pressor effect, which then per-
sisted though the quantity of ether administered was reduced

4 *Rabbits without anæsthesia* When histamine is injected, in a dose
of 0 02–0 05 mgm , into the lateral vein of one ear, the other ear can be
seen to flush after a few seconds The wide spontaneous variations in
the vessels of the rabbit's ear make it difficult, however, to be certain of
the significance of such an event One ear was, therefore, denervated
completely in each of three rabbits, by removing the superior cervical
ganglion and cutting both the great and small auricular nerves This
operation was performed under deep anæsthesia with ether and with
complete aseptic precautions When the small wounds had healed
completely, and sufficient time had elapsed for complete degeneration
of the nerves, these rabbits were then used for observations with hista-
mine The vessels of the denervated ear show some variation in calibre,
constricting if the rabbit is excited. Their tone, however, is much more
stable than that of the vessels of the normally innervated ear, showing
none of the normal spontaneous rhythm[6], and the changes produced
in response to histamine are unmistakable The ear was carefully shaved,
and spread out for observation in front of an opal glass electric light

bulb The number and width of the visible venules was noted, and also the general colour-tone of the tissues between the visible vessels A dose of histamine (0 03–0 05 mgm) was then injected into the lateral vein of the other (normal) ear The first visible effect, following the injection after 5–10 sec , was a narrowing of the central artery of the ear Almost simultaneously it could be seen that the number of visible venules had increased and that those previously visible were fuller, while the general colour of the tissues between the visible vessels had become notably redder Both effects—arterial constriction, and capillary dilatation with widening and filling of the veins—were visible in all cases, and followed every injection Their relative prominence, however, varied In some instances the arterial constriction was severe, and the general reddening and venous filling correspondingly small, in others the arterial constriction was slight, and the general dilator effect very pronounced The dilatation lasted for 30 to 60 sec , and then faded The character of the reaction, with respect to the predominance of arterial constriction or capillary dilatation, seemed to be fairly constant in the same rabbit, the ear which showed a very pronounced dilator reaction at one injection would, apparently, give a similar reaction at each succeeding injection.

DISCUSSION

It will be seen that the results of direct observation of the changes in the ear-vessels support the assumption, made to explain the varying effects on the arterial pressure, that histamine has a dual action on the rabbit's vessels—a constrictor effect on the visible arterial branches, and a more peripheral dilator effect, spreading on to the capillaries This brings its action on the rabbit's vessels into line with what was previously known of its action on those of the cat In both cases we have a less peripheral constrictor and a more peripheral dilator action. The striking differences apparent between the action of histamine in these two species, under the usual conditions of experiment, are evidently the result of the relative prominence of these two effects, and the way in which they are modified by the influence of the anæsthetic In the cat ether appears rather to enhance than to depress the capillary-dilator effect of histamine The tone of the small vessels of the cat under ether is usually very strong, and the arterial pressure correspondingly high Under such conditions the effect of histamine has the aspect of a pure vaso-dilatation, resulting in a simple fall of blood-pressure, pronounced in degree, and still perceptible with extremely minute doses Only with large doses of histamine does arterial constriction complicate the vascular effect in

this species The capillary tone in the rabbit appears to be normally weaker, and to be readily depressed by such anæsthetics as ether and urethane Histamine in this species produces no visible effects until the dose is raised to a point at which arterial constriction complicates the effect, and often becomes so predominant as to obscure altogether the effect of the more peripheral dilatation That the latter nevertheless exists, and may under appropriate conditions determine the effect of histamine on the arterial pressure and on the resultant vascularity of a tissue, is demonstrated by the results here recorded It may be added that the sudden injection of measurable quantities of histamine into the general circulation cannot be taken to represent, even crudely, the manner in which it may be supposed to come into action under physiological conditions Assuming, as much recent evidence entitles us to do, that histamine is liberated from the cells of the tissues under various conditions of stimulation (2, 3), it is on the capillaries in immediate relation to those cells that its first effect would be produced Only under quite abnormal conditions can it be supposed that sufficient histamine would escape into the general circulation to affect the arteries directly

My thanks are due to Dr H H. D a l e for help and advice throughout the experiments

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EXPERIMENTS ON VISCERAL SENSATION

Part I The relation of pain to activity in the human œsophagus

By W W PAYNE AND E P POULTON[1]

(From the Physiological Laboratory and the Medical Investigation Department of Guy's Hospital, London)

In a previous communication(1) we described the movements of the œsophagus, stomach, duodenum and jejunum associated with the sensation of pain It was found that pain in the epigastric angle was associated with activity of the œsophagus, and that the latter occasioned the retrosternal pain commonly called "heartburn" These observations were the starting point of the present investigation

1 METHODS

Our apparatus is drawn in Fig 1 An ordinary toy balloon of about 50 c c capacity (i e outside the body it was large enough to hold up to 50 c c of air without causing any tension on its walls, and consequently any increase of pressure inside it) containing a lead weight was tied on to the end of a thin rubber gastric tube, as described in our previous communication. A special note is made where bags of other capacities were used The gastric tube was connected by means of a T piece to a funnel and a manometer, the other end of the manometer was connected to a Brodie's bellows which recorded on a kymograph All air was removed from the bag before swallowing It was located in the œsophagus by measurement or by means of X-rays, which showed up the lead weight at the bottom of the bag, and the small metal connecting tube over which the rubber bag was tied The bag could be filled rapidly with air by opening the clip above the funnel after forcing into the latter the required volume of water The manometer was connected to the Brodie's bellows so that the record on the kymograph represented the actual pressure in the œsophagus in cm of water It was a simple

matter to calibrate the bellows beforehand by marking the level of the
lever with the manometer at zero, and again when the manometer
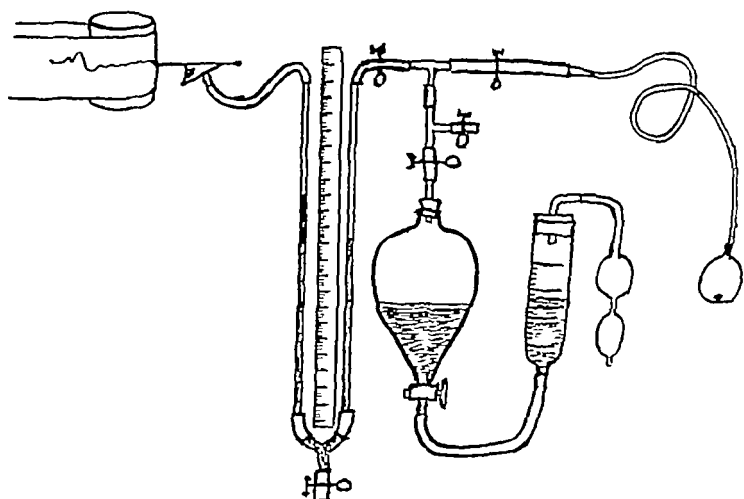
Fig 1

registered a pressure of 40 cm   Sometimes other pressures, *e g* 20 cm ,
were also marked However, as shown in Fig 3, the height of the lever was
not exactly proportional to the volume in the bellows, so that with only
two pressures marked it is not possible to read accurately the absolute
value of intermediate pressures Occasionally the height of a big con-
traction was read off directly from the manometer So far as we can tell,
previous observers who have used a bag method for recording œso-
phageal movements have blown up the bag tight *in situ, i e* they have
forced in more air than could be accommodated in the bag outside the
body without stretching its walls If this is the case the pressure of the
manometer will give no true record of the pressure actually exerted by
the œsophageal wall In our experiments we purposely introduced less
than the 50 c c capacity of the bag Consequently the pressures that
we have recorded may be taken to represent accurately those excited
by the œsophageal wall in its attempt to elongate the bag, since the
latter is of course spherical when distended with 50 c c air outside the
body

Boldireff[3] has criticised the balloon method of investigating
movements of the empty intestines, because the presence of the balloon
itself is sufficient to excite movements Carlson is inclined to agree
with him We should like to state emphatically that in the case of man

at any rate this criticism does not hold good for our technique For instance, in our previous paper we showed that the presence of a small balloon in the duodenum does not necessarily cause contractions, and our present work indicates that an air bag in the œsophagus, containing only 5 or 10 c c air, often does not excite any movement at all after it has been *in situ* for a few minutes

2 THE MOVEMENTS OF THE ŒSOPHAGUS

Kronecker and Meltzer(2, 4) found in records taken by means of air balloons in the pharynx and œsophagus that swallowing caused an immediate sharp rise of pressure (p in Fig 2) which coincided with the rise of the larynx and passed with extreme rapidity down the œsophagus After this wave there was a fall in pressure (n) and then a much larger rise which was due to the peristaltic wave, excited by the act of swallowing They also showed that an eructation was followed by a peristaltic wave down the œsophagus without any rise of the larynx They mentioned the presence of other peristaltic waves not associated with swallowing, but they did not enter further into this subject Cannon and Washburn(6) described movements in the œsophagus during hunger which they argued would probably coincide with the hunger contractions of the stomach Carlson and Luckhardt(7) proved that this was the case In addition to peristaltic waves Carlson and Luckhardt described local contractions of two kinds (1) waves occupying less than 2 sec duration, (2) local alterations in tone
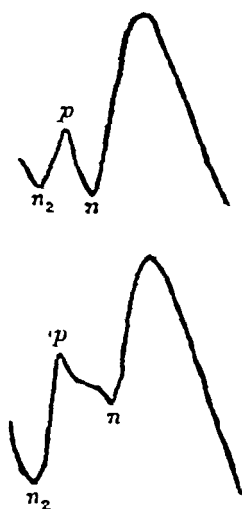
Fig 2   Diagrams of œsophageal waves due to swallowing The lower shows a rise in diastolic pressure due to an increase in tone

We have only occasionally met with Carlson and Luckhardt's "two seconds" waves They can be seen in the lower tracing in Fig 3 We have most commonly found a wave of about 4 sec duration For instance, in the first part of Fig 8 nine of these waves were counted in 40 sec , but the rate is variable Fig 4 A shows that they are local, since they are prominent at the beginning of the upper tracing, but are hardly present at all in the lower one

Danielopolu, Simici and Dimitriu(8) have suggested that peristaltic waves are always associated with swallowing, but we cannot agree with this We made a special study of this question, since in all our

experiments the subject signalled when he was conscious of swallowing voluntarily The type of peristaltic wave resulting was like that described by these authors But a similar wave was often recorded without the subject being aware that he had swallowed at all, this was evidently similar to what Hurst(5) described and to the secondary peristaltic wave of Meltzer(9) and to the peristalsis that follows an eructation described by Kronecker and Meltzer The similarity of the peristalsis of swallowing and secondary peristalsis is shown in Fig 4 B, which shows six similar peristaltic contractions, the fourth one alone followed a swallow

In our previous communication we described a phenomenon allied to swallowing to which the term "secondary swallow" might be applied It consists of a sensation at the back of the throat often, but not always, accompanied by a slight movement of which the subject is just conscious, but there is no obvious rise of the larynx It is perhaps the same as the "small swallow" of Miller and Sherrington(24) Secondary swallowing is followed by the same wave of peristalsis as follows a true swallow Examples are shown at the end of Fig 6 A

Danielopolu and his colleagues regarded the n-depression as being due to negative pressure produced in the œsophagus from the rise of the larynx at the moment of swallowing, and they stated that this wave was not so obvious in the lower part of the œsophagus as in the upper part Such a wave was not always demonstrated well by Kronecker and Meltzer, or by ourselves, though in our previous paper we noted its presence The reason is that from the beginning of the act of swallowing there is often a gradual rise in the "diastolic pressure" of the œsophagus, $i e$ the pressure in the œsophagus, between the various contraction waves, as is shown in the last swallow in Fig 6 A Consequently the n-depression, though present, is often at a higher level than the tracing just before it We have, however, now obtained clear proof that Danielopolu's suggestion is incorrect, and that the wave is really due to inhibition, which precedes the peristaltic contraction, as was inferred by Kronecker and Meltzer after studying repeated swallows We have, in fact, succeeded in demonstrating it in an exaggerated form at the bottom of the œsophagus

Exp 1 (Fig 3) May 9th, 1923 Subject W W P Two air bags were placed in the œsophagus the lower one had a capacity of 70 c.c The upper one was tied so as to be kept immediately above this bag and it contained 5 c.c. of air Records were obtained from both bags On putting 70 c.c. in the lower bag which had previously been empty, an involuntary swallow was taken, and the pressure in both bags was at once increased although the increase was slower in the upper bag Pain of moderate severity was experienced immediately Minor variations in pain were experienced but not recorded

L, M, N, R are simultaneous points on the tracings and represent the apices of peristaltic waves. The wave *D* resulted from clearing the throat (secondary swallow) and *R* from
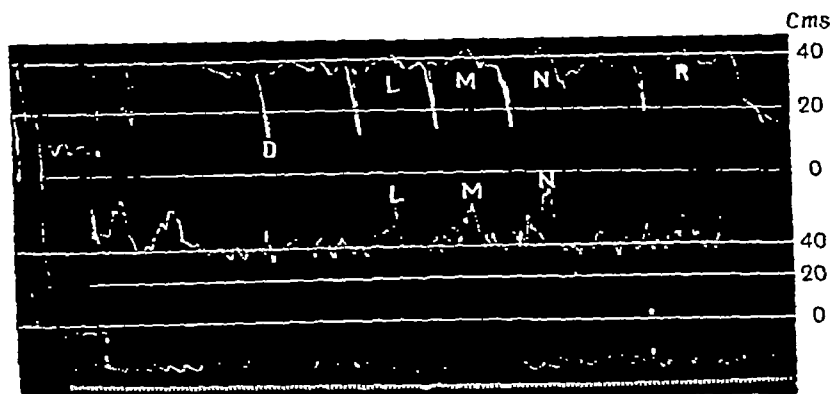
Fig 3 *Exp* 1. Upper two tracings form a double œsophagogram Pressure shown in cm. water Third tracing is respiration. Inspiration is shown by an upstroke on the respiratory tracing and a down stroke on the œsophageal tracing Time in seconds Simultaneous points are shown by small white strokes. All other figures are arranged similarly

swallowing The large depressions in the upper tracing precede the peristaltic waves, and can only be due to temporary sharp relaxations of the muscle very obvious because it was previously strongly contracted. The bottom of the lower bag was 13 inches below the teeth, in a position where, on the theory of Danielopolu and others, the *n*-depression can only be very slightly marked.

Our observations suggest an application to the œsophagus of Bayliss and Starling's law of the intestines or "myenteric reflex," which states that stimulation causes dilatation below and contraction above the point stimulated(10)

Alvarez(11) in recently criticising this law suggested that the wave of inhibition preceding a peristalsis depended for its existence on the state of the muscle—whether it was fatigued, contracted, relaxed, etc Our observations, as also one of his (see Fig 2 A, p 236) show that the muscle must be in a state of contraction for the wave to be marked

We have further evidence of the application of this law also shown in Fig 3 The immediate result of blowing up the bag was to cause at the position of the bag a rapid rise of pressure not lasting for more than half a second from the mechanical stretching of the muscle, but this was followed immediately by relaxation with lowering of pressure, while in the position of the upper bag the muscle contracted continuously for one or two seconds In other words, blowing up the bag caused relaxation at the spot and contraction above

A V Hill(12) has recently shown that suddenly stretching plain denervated muscle causes at first relaxation   Is the relaxation in our experiment an example of this generalisation?   It is interesting to observe that the relaxation was also present in the bag above though it began some seconds later, showing that it was transmitted up the œsophagus   There is a better example of this in Exp 2

Exp 2 (Fig 4 A) May 1st, 1923   Subject W W P   Two bags in the œsophagus, one just above the other   The upper bag contained 8 c c   40 c c air were introduced suddenly into the lower bag previously empty, a series of secondary peristalses resulted. Between each peristalsis there is one of our ' 4 sec " simple or local contractions   Slight continuous pain was felt with exacerbations coming on at intervals and ending off somewhat indefinitely

In this experiment the relaxation of the upper bag only lasted 1 sec and then the original wave of contraction was continued, reaching its maximum 5 sec from the introduction of the air   This formed the peak of the first peristaltic contraction, which passed down and affected the lower bag at the end of the relaxation brought about by the sudden stretching

Kronecker and Meltzer considered that the p-wave was due to the impact of saliva on the bag, but in our double tracings (Fig 4 B) the p-wave is very well marked in the lower tracing, when the presence of the upper bag would have prevented the impact of saliva on the lower one   The more probable explanation is that at the beginning of a swallow a momentary positive pressure develops in the pharynx which passes rapidly down the œsophagus, and this may be responsible for the rapidity with which liquid food is shot down to its lower end

There is not infrequently a fall in pressure in front of the p wave, which may be called the n_2-depression   Danielopolu and his colleagues have regarded this depression when it occurs as being identical with the n-depression neglecting the latter altogether (see Fig 12 of their paper)   They did not take respiratory tracings, but in our later observations we always recorded the respiratory movements by tying a band round the chest, placing a rubber ball inside it, and connecting this with a piston recorder   It was found that negative depressions in the œsophageal tracing were often respiratory in origin   Thus, in one experiment, after a period of apnœa produced by forced breathing, a gulp was taken in an attempt to swallow quickly   At the position of the gulp there was a very large inspiration which exactly coincided with a large n_2-depression   The latter was followed by the p-wave, the n-wave, and the wave of peristaltic contraction   Miller and Sherrington have shown that swallowing inhibits respiration   We have found that even secondary

peristalsis may have an effect Thus in Fig 4 A the inspiration that precedes each secondary peristalsis is shallow, but earlier in the tracing secondary peristalsis had no effect on respiration at all.

Fig 4 A. *Exp* 2. Part of a double œsophagogram, showing relation of respiration and pain to peristalsis Descent of signal indicates exacerbation of pain.

Fig. 4 B. *Exp* 3 Double œsophagogram. Rise of lever indicates pain. The arrows show where a swallow was signalled.

Summarising we may say that swallowing causes (1) an immediate rise in diastolic pressure (caused by an increase of tonus, as dealt with

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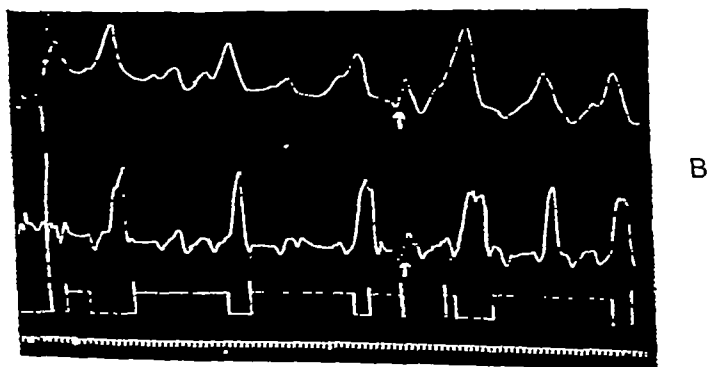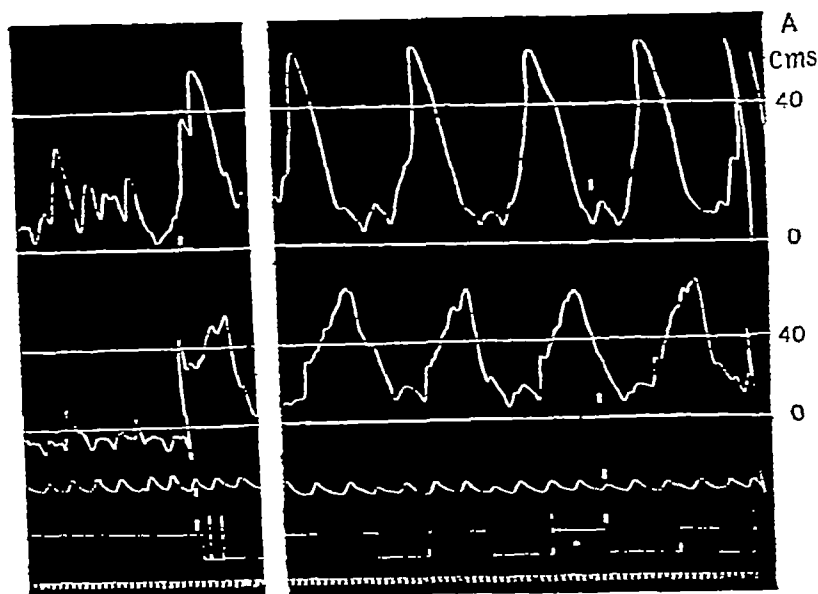Fig 4 A *Exp* 2 Part of a double œsophagogram, showing relation of respiration and pain to peristalsis. Descent of signal indicates exacerbation of pain.

Fig 4 B. *Exp* 3 Double œsophagogram. Rise of lever indicates pain The arrows show where a swallow was signalled.

Summarising we may say that swallowing causes (1) an immediate rise in diastolic pressure (caused by an increase of tonus, as dealt with

later), (2) a small rapidly travelling wave of positive pressure followed at an interval, the length of which depends on the part of the œsophagus being investigated, by (3) a negative wave, and immediately afterwards (4) the main contraction wave due to peristalsis But respiratory irregularities of themselves often produce small waves so that analysis from an œsophageal tracing alone is not always possible

3 THE EXPERIMENTAL PRODUCTION OF PAIN IN THE ŒSOPHAGUS

The first effect of increasing the volume of air in a balloon inside the œsophagus is to cause repeated peristaltic contractions to pass down When the air put into a bag of 50 c c capacity reaches 20 c c recurring pain of quite short duration may be noticed This was marked on the kymograph tracing in an experiment on W W P , Dec 21st, 1921 (Fig 5 A) It will be seen that the sensation of pain was recorded during re laxation, *i e* after the œsophagus had contracted to the maximal extent It might be argued that the sensation was really due to the contraction on the bag, but that the delay in its appreciation, or in the signalling, caused it to be recorded too late That this explanation was not correct was shown on another occasion

Exp 3 (Fig 4 B) Subject W W P Two bags in the œsophagus. The upper bag con tained 25 c c of air and the lower bag 20 c c In this subject it was found by X rays that the cardia and the xyphisternum were on the same level, and the cardia was 17½ in below the teeth The top bag was situated 1 in below the suprasternal notch, *i e* 7 5 in from the teeth, while the lower bag was situated 6 in lower There was an interval of 2 8 in between the bags The fourth wave on the tracing was due to a swallow, and this has already been dealt with. Pain was felt at the suprasternal notch and at the xyphisternum The upper pain began first, and the lower pain ended last Consequently the beginning of the painful periods in the tracing should be compared with the upper tracing and the end with the lower one It will be noticed that pain was signalled during the whole period between contractions, but not during the contraction itself Furthermore, the duration of the pain and its absence bear a quantitative relation to the period between the contractions and the period of the contraction itself If the pain was due to contraction, but was signalled late then the period of the pain would still correspond to the length of the contraction which is not the case The explanation that we have already put forward of this result (13) is that the bag caused pain by stretching the œsophagus while the contractions relieved the stretching and so abolished the pain Furthermore, Fig 4 B shows that these contractions are peristaltic in origin since each contraction in the upper curve is also shown in the lower curve On measuring it is found that there was a delay of about 2½ sec between the two contractions at the beginning of the tracing and 1½ sec at the end which indicates the time taken for the wave to pass from one bag to the other A similar result was obtained in *Exp* 2 (Fig 4 A)

It became of interest to devise an experiment in which the peristaltic wave would be unable to compress the bag, as Sir Henry Head informed us that he had observed in certain patients that pain was

expenenced dunng the cóntractions of the bladder if there was a stnc-ture, and no unne was passed. For this purpose we enclosed a rubber bag
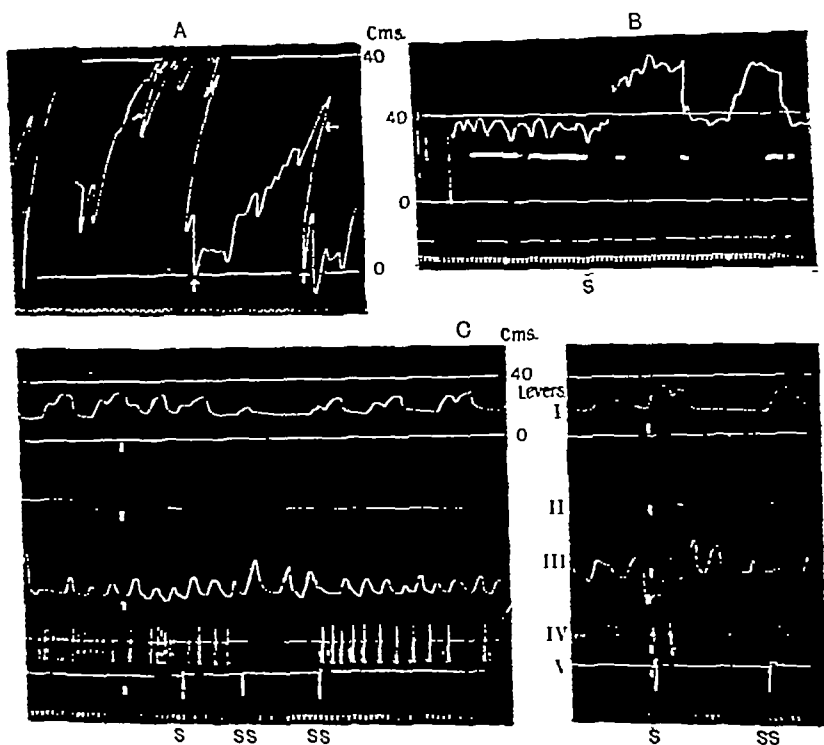
Fig 5 A. Subject W W.P No note of volume m bag Pam signalled three times dunng relaxation. Beginning and ending of pam marked by arrows.

Fig 5 B. Subject W W.P 45 c c m bag The signal marked *S* indicates a swallow Pam was felt contmuouslv Penods of severe pain are indicated by honzontal lines beneath the tracmg

Fig 5 C. Subject E.P.P *Exp* 6 Lever I—œsophageal tracmg Lever II shows the level of the Hg manometer attached to the mcompressible bag Lever III—respiratory tracmg Lever I\—pam. Lever V—swallows (*S*) and secondarv swallows (*SS*) The Hg manometer read 23 8 and later 23 1 cm m the first part of the tracmg The second part shows pressure waves m both bag and manometer tracmgs. The sharp nse was due to a cough.

about the size of a large finger-stall mside an mextensible lmen case The bag and case were tied tightly on to a stiff catheter, and water could be forced mto the bag so that such a high pressure was produced mside as to preclude all possibility of compression by the œsophageal muscle

Exp 4 (Fig 6 A)   June 22nd, 1922.   Subject W W P   The incompressible bag on a stiff catheter was placed in the œsophagus with its end about 14 in. from the teeth.
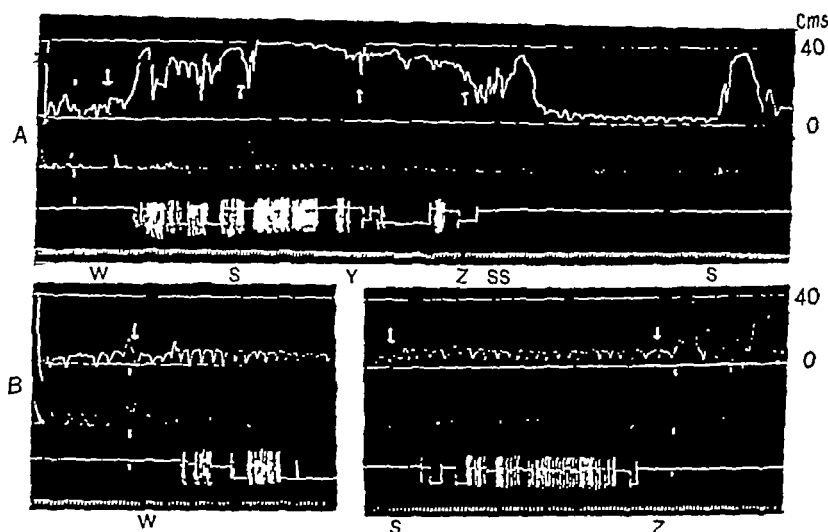
Fig 6 A. *Exp 4* Œsophageal movement above an incompressible bag   *S*, swallow, *SS*, secondary swallow, each followed by a peristaltic contraction.

Fig 6 B *Exp 5* Œsophageal movements below an incompressible bag The middle part of the experiment is omitted.

A second rubber bag, containing 10 c c. of air and arranged according to our usual technique, was kept just above the incompressible bag with its lower end 11¼ in. down, in order to record the contractions just above the incompressible bag   During the preliminary period the pressure between the contractions (diastolic pressure) was 2 to 4 cm., and the pressure at the height of a peristaltic contraction (systolic pressure) was 32 to 33 cm Then at W about 17 c c. of water were suddenly forced into the incompressible bag A series of contractions began and pain was felt at once it was continuous although it varied in intensity It was worse during the contraction, and was so bad that it was impossible for the subject to keep the lever down all the time He found it easier to move it up and down the rapidity of the movement indicating the extent of the pain. At a point marked *S* he swallowed experimentally and the pain became still worse, so that eventually a little water—about 5 c c —had to be let out this is marked by the depression at *Y* The diastolic pressure rose at the beginning of the experiment to 7 cm of water, and this pressure was recorded twice Fourteen seconds later the negative wave of the voluntary swallow showed a pressure of 12 cm so that the diastolic pressure was gradually rising (The small depression 1 sec later was due to some movement of the body owing to the pain, and has its counterpart on the respiratory tracing) The effect of the swallow was to cause a continuous contraction of 27 sec. duration—a regular tetanus—at a pressure of 40 cm The water was let out at *Z* and after a secondary swallow (*SS*) the œsophagus was quite quiet showing only respiratory movements The secondary swallow and a voluntary swallow (*S*) taken later on produced no pain at all

We wondered whether in this experiment the tetanus was caused by a peristaltic contraction not being able to get past the incompressible bag This point was specially investigated

Exp 5 (Fig 6 B) July 3rd, 1922 Subject W W P The air bag was passed down the œsophagus to a distance of 14 in., while the water bag on the catheter was placed 2¼ in higher up, so that the two bags did not overlap one another On forcing water into the upper bag at the arrow marked *W*, continuous pain was noticed. Probably the bag was not filled up quite so tightly as last time. After swallowing (*S*) the pain was a little less to begin with, and then worse a few seconds later There was no effect on the lower bag in spite of the extreme activity that had been shown to be present above the incompressible bag During the preliminary period the diastolic pressure below the incompressible bag was mostly below zero During the experiment it was most of the time between 5 and 3 cm The movements were respiratory At the end of the experiment when water was let out (*Z*) the diastolic pressure fell to as low as 0 5 cm , the bag now recorded two peristaltic contractions, so that these contractions had clearly been prevented from travelling down by the presence of the incompressible bag

In another experiment the pressure in the incompressible bag was measured

Exp 6 (Fig 5 c) Feb 22nd, 1923 Subject E.P P An incompressible bag on a catheter was placed in the lower œsophagus, and just above a rubber bag containing 10 c.c The incompressible bag was connected with a mercurial manometer which also recorded on the tracing Pain of varying intensity was felt throughout the experiment. The pressure in the incompressible bag was varied from 24 to 22 cm. of mercury It remained perfectly level for 3 min. at a time, which showed that the œsophageal muscle had no power against a pressure of this magnitude During all this time a continuous series of contractions occurred above the incompressible bag The severity of the pain was roughly graded by the frequency of movement of the pain lever It was less in the middle of the tracing when the diastolic pressure in the upper bag was lowest. Towards the end of the experiment, as shown in the second part of Fig 5 c, small waves in the tracing from the mercurial manometer were recorded and these followed shortly after the contraction in the upper bag On removing the catheter it was found that the stitches had given way in one place, and part of the rubber bag had herniated through its linen cover On this account the bag became slightly compressible so that contractions were recorded. The experiment suggests that the linen bag was being spasmodically compressed all the time, but that it was only towards the end of the experiment that the compressions could be recorded.

It was considered advisable to try and get radiographic evidence of the appearance of the œsophagus while pain was being produced experimentally An air bag was made out of two rubber balloons of the same size by squeezing one inside another The space between the two was filled with an emulsion of bismuth and the necks tied one over the other on to a rubber catheter over a piece of metal tubing in the ordinary way On filling the inner balloon with air the bismuth was spread over its surface and an air bag was obtained with its wall opaque to X-rays

Exp 7 (Fig 7) March 1st, 1924 11 a.m. Subject W W P This subject, who has always been inclined to dyspepsia, had had no symptoms for 5 days. Two catheters fitted with

opaque walled air bags were passed into the œsophagus, one above the other, either one or
the other was connected with a water manometer   When each contained 10 c c of air the
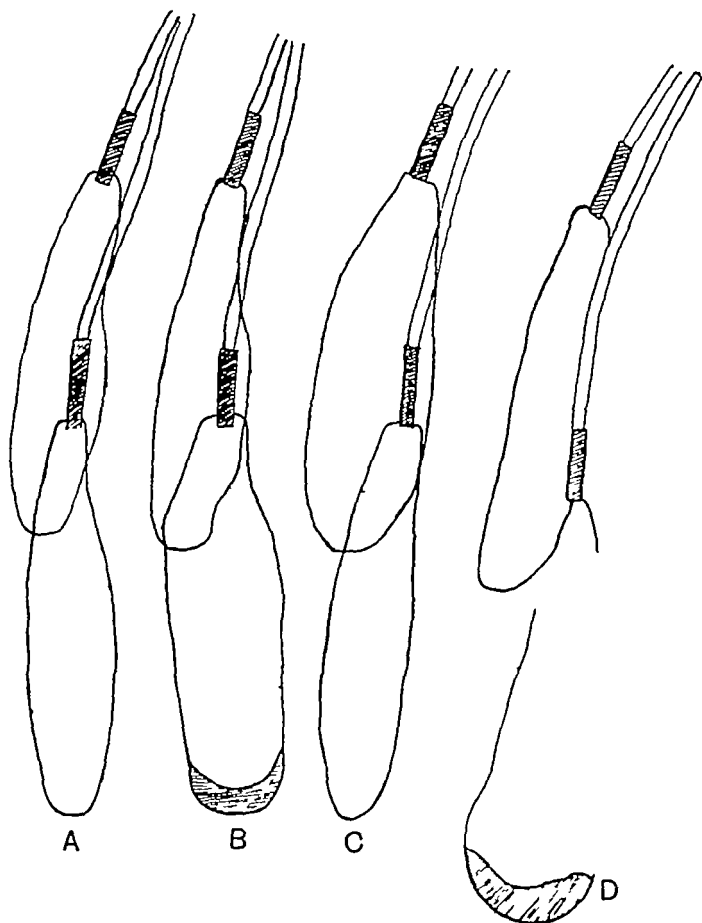
Fig 7   Diagrams of two air bags in the œsophagus.

patient experienced a small amount of continuous pain. *Fig 7 A is a tracing of the air bags
in situ* The walls were well defined, indicating that during the two or three seconds ex
posure the œsophagus had remained fairly quiet The lower bag was then filled with 30 c.c.
of air (B)   Pain of medium intensity was experienced The lower bag was dilated but not
lengthened The upper bag was practically unchanged   High pressures were observed in
the manometer to which it was attached but it was not found practicable to record them
In the next observation (C) the lower bag contained 10 c c while the upper bag contained
40 c.c. The pain was described as nearly maximal in intensity The shadows were not
perhaps quite so sharply defined as before Measurement showed that the upper bag was
4 mm longer than before while the length of the lower bag was not altered. This suggests
that a peristaltic wave had reached the upper bag but had not affected the lower one *In*

the next observation (D) 10 c.c. were again put into the upper bag, and 40 c c into the lower bag A maximal pain was experienced about 1 sec before the exposure of the plate began. It then began diminishing and was nearly, but not quite, minimal by the end of the exposure In the plate it was almost impossible to see the lower bag at all, except for the dark shadow of the bismuth in its lower end which lay beneath the dome of the right diaphragm. The lower bag was 1 cm longer than previously, while the upper bag was 9 mm longer It is evident that the pain was becoming less just as a peristaltic wave was passing down over the bags causing them to be elongated, and therefore compressed. We have in other experiments observed the same phenomenon, both in the case of E P P and W W.P , using a rather different technique

In our preliminary communication (13) we suggested that the cause of the pain during contraction on an incompressible bag was that the end organs were being pressed tight against a hard surface but that in contra-distinction to this when the foreign body was a rubber bag containing air the pain was due to stretching Further work, and in particular Exp 1, caused us to alter our views on the subject Even though the foreign body which in this experiment gave rise to the pain was an air bag rather than an incompressible bag, the upper tracing showed a considerable resemblance to the tracing obtained above the incompressible bag in Exp 4, suggesting that the cause of pain in these two experiments must be the same

A simple explanation might be that an isometric contraction on the incompressible bag was painful But on the analogy of skeletal and uterine muscle this is very unlikely, for instance, there is no pain on attempting to flex the forearm against resistance, and the intermittent contractions of the uterus that occur throughout pregnancy are not painful

4 TONUS IN PLAIN MUSCLE

Before considering the factors causing pain it is necessary to discuss the meaning of the term posture and tone when applied to an organ like the oesophagus at its lower end, the walls of which contain plain muscle Sherrington (13) has pointed out that a viscus, such as the bladder, does not behave like an elastic bag, which shows a gradual increase of pressure with increasing tension of its walls as its content of fluid increases, but varies in posture, so that it can accommodate an increasing volume of urine while the pressure inside is not necessarily increased, although alterations in pressure due to contractions and relaxations of the wall do take place continually as the bladder becomes full. Thus A R Thompson (14) found with 250 c c of liquid in the bladder that the maximum systolic pressure, *i e* the maximum pressure of one of these contractions, during a period of a minute or two, was 14 2 cm. of water,

while the minimum diastolic pressure was 9 4 cm   After an interval of
10 min   the whole pressure in the bladder had increased, the maximum
systolic pressure now was 26 8 cm   while the diastolic pressure was
14 8 cm   A still greater pressure was observed with 500 c c , while the
difference between systolic and diastolic pressures was also considerably
increased   The following experiment shows in a general way that the
behaviour of the plain muscle in the bladder and œsophagus are
similar

Exp 8 (Fig 8)   May 22nd, 1922   Subject E P P   A bag containing 10 c c. of air was
kept in the lower end of the œsophagus for over 5 min.   The diastolic pressure varied
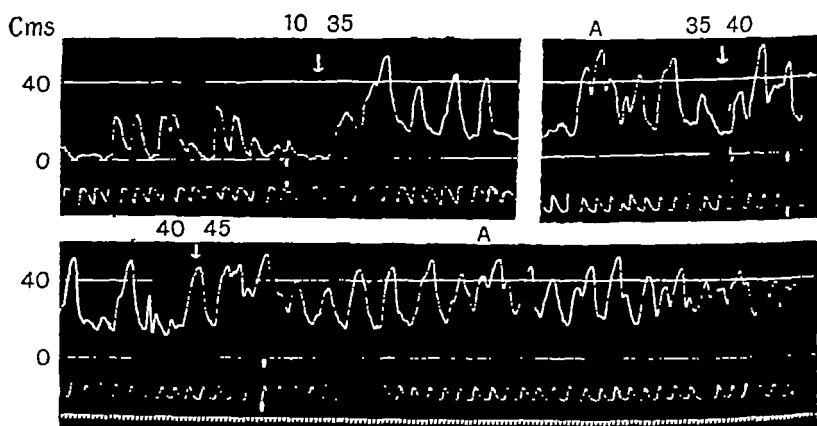
Fig 8   *Exp* 8   Sections of a tracing to show the effect of increasing the volume of air in
      an œsophageal bag   The increase took place at the arrows, the volumes being given
      above the tracing

between 0 and 5 cm   of water   Contractions giving a systolic pressure of 28 cm. appeared
at intervals, although there was a period of 34 sec   when only cardiac and respiratory
movements were recorded. The bag was then filled with 35 c c. of air for a period of 130 sec.
The diastolic pressure varied between 9 and 15 cm   The maximum systolic pressure was 54
Next with 40 c c   of air, during a period of 84 sec , the diastolic pressure varied between 11
and 17, with a maximum systolic pressure of 55 cm   while with 45 c c , for a period of
146 sec , it varied between 14 and 25 with a maximal pressure of 53   Through all this experi
ment the diastolic pressure gradually increased, but the height of the contraction remained
about the same in the later stages, so that the excursions of the manometer became smaller
A similar diastolic rise and constant systolic plateau have been observed in the stomach
by A. J   Carlson (21) during the course of prolonged hunger   The diastolic pressure may
be said to form a base line and the difference between this and the maximum pressure
recorded during systole is analogous to the pulse pressure   Another feature of interest in
many of our tracings was best seen on this occasion when 45 c.c   of air were introduced.
This was a gradual rise and fall in the diastolic pressure, a period as long as 50 sec   some
times elapsing between successive waves.

It is clear that the œsophagus resembles the bladder in the increasing activity shown as the amount of its contents increases But Fig 7 shows that, where the volume of air in the bag is increased from 10 to 30 or 40 c c , there is some increase in the posture of the œsophagus as indicated by the diameter of the bag Therefore, in the œsophagus increased activity is associated with increase of posture, as is also the case with the bladder, though obviously the bladder can undergo a much larger increase in posture than the œsophagus can

Like the bladder, the œsophagus requires time for postural adaptation, since with a given volume of air in the bag a lower diastolic pressure is recorded if the air has been introduced gradually than if it has been introduced suddenly Thus on one occasion with the subject W W.P the bag of 200 c c capacity containing 15 c c air was kept in the œsophagus for 3 min The content was then increased to 60 c c , and the following series of diastolic pressures were recorded, 10, 11 5, 12, 10, 32 10 cm during a period of rather under 3 min The bag was then emptied and 60 c c of air were introduced at once The diastolic pressures recorded were 50, 51, 36, 35, 31, 29 cm during a period of 2 min

Carlson has assumed that tonus in plain muscle is measured by the value that we have defined as diastolic pressure We would suggest that tonus should be defined as the *tension* in the muscle during diastole, and this will depend not only on the pressure but on the radius of curvature, since when the pressure (p) in a hollow organ is due to tension (T) of its walls, the following relations hold(15), viz $T = 2\,pr$ for a sphere, and $T = pr$ for a cylinder

TABLE

	Average diameter of upper bag cm.	Volume of air in upper bag c c.	Volume of air in lower bag c.c
A	2 25	10	10
B	2 35	10	30
C	3 00	40	10
D	2 08	10	40

It is possible to make an approximation to the relative values of the tension of the muscle in diastole and systole from the photographs of the two rubber bags *in situ* in Exp 7 (see Table) The diameter given is the mean of two measurements across the upper bag at the upper and lower level of the lead tube belonging to the lower bag

It is clear from our other experiments that the pressure in the upper bag of *C* must have been greater than it was in *A*, since the volume of contained air was greater, but the diameter was also greater, and so the tension must have been greater still In this case it would be incorrect

to regard the tension or tonus of the muscle as being measured solely by the pressure

On the other hand, in A and B the diameters of the two upper bags were very much the same but in B, owing to the larger volume of air in the lower bag, the pressure in the upper bag must have been higher than it was in A (see Fig 3) Hence in this case it is permissible to regard the tension or the tonus as being measured by the pressure

During systole, however, the conditions were different because D shows that the peristaltic wave had lengthened the upper bag and narrowed its diameter a little as compared with B Both the tension of the muscle at the upper bag in D and the pressure exerted must have been greater than in B, but in this case the increase of tension must have been less than the increase of pressure

To sum up, it follows from our definition of tonus that with the diastolic pressure constant an increase in the volume of the bag will result in an increase of the tonus owing to the increase in the diameter of the viscus If, as is usual, the diastolic pressure also increases this would indicate an even greater increase in the tonus If the volume remains constant, variations in tonus will not cause a measurable change in the diameter of the bag, and in this case the diastolic pressure will be a measure of the tonus The increase of tension of the muscle during the progress of a peristaltic contraction is relatively less than the increase of pressure exerted by it

It is interesting to note that the only way in which a peristaltic contraction can cause compression of a rubber bag containing air is by making it lengthen so that the radius of curvature of the muscle simultaneously diminishes The application of Boyle's law shows that an increase of 40 cm in pressure will only cause a diminution in the volume of air in the bag by 4 p c or if the bag is assumed to be a cylinder of under 2 p c in length of the radius

It might be objected that "tonus" as here defined merely represents the tension in the passively stretched muscle But we have already shown that the mechanical effect of sudden stretching is momentary (Fig 3), and the subsequent alteration in diastolic pressure must be due to activity on the part of the muscle, since it is constantly varying Further, after a bag in the œsophagus is filled with air or water a continuous increase of diastolic pressure, and so tonus, takes place above the bag (Figs 3 and 6 A), and to a very small extent even below (Fig 6 B) These changes must be due to active contraction of the muscle wall, and not to its being stretched mechanically by the air bag

Variations in tonus occur while the volume of air in the bag is kept constant Fig 8 shows how large they may be, for the triple contractions marked at *A* may be regarded as made up of three separate contractions, the last two of which begin from a position of high tonus, the diastolic pressures having risen to nearly 40 cm We have found that occasionally tonus exerts pressures up to 50 or 60 cm On comparing the pressure tracings from the œsophagus of the two subjects W W P and E P P there was this striking difference that, whereas in the case of W W P single large contractions were often recorded when the tonus remained rather low, in the case of E P P the contractions were multiple and the tonus increased, so that the contractions were never very large, while the maximum systolic pressures were much the same as with W W P Radiograms of the lower part of the œsophagus taken immediately after swallowing a mouthful of barium emulsion showed that the maximum diameter was for E P P 2 4 cm and for W W P 2 cm Perhaps this is partly responsible for the difference in behaviour

Sherrington(14) and Bayliss(16) have described the two functions of a muscle as contraction and posture The latter function is sometimes supplied by special muscles which are called "check" or "catch" muscles since they fix the posture We would suggest that as far as the œsophageal plain muscle is concerned tonus, as we have defined it, has a check function in relation to contraction, since when the tonus rises during a contraction it checks the relaxations, and allows the tension to fall in a series of steps The experiment on E P P in Fig 8 provided a very good example of this check action, and this is a great contrast to the experiment shown on W W P in Fig 4 A

5 A THEORY OF VISCERAL PAIN

In a previous section we dealt with the factors which caused an increase and diminution in pain produced artificially in the œsophagus, and the suggestion was made that pain was due to stretching of the wall

Our development of this theory is as follows A foreign body such as an air bag or water bag becomes a stretching force, as soon as its circumference begins to exceed the circumference of the surrounding œsophagus This leads to a state of tension in the wall The latter contains muscle fibres, sensory end organs and the free endings of nerve fibres that subserve the sense of pain All these structures take up the tension If a nerve ending consists of a longitudinal sheath enclosing liquid or

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semi-liquid contents, the tension may act by causing stretching of the sheath and deformity of its contents This process causes pain

Since a rise of tension in the walls of a hollow viscus is often associated with a rise of pressure inside it, we should expect to find pain associated with a rise of diastolic pressure The following observations show that this is the case

When a bag is slowly filled with air the diastolic pressure rises and at a certain point pain is experienced For instance, in the case of W W P on May 25th, 1922, with 10 c c in a bag of 50 c c capacity the diastolic pressure was − 5 0 cm , and there were no contractions With 20 c c the diastolic pressure was 2 cm , and the systolic 67 cm With 30 c c the diastolic pressure varied between 11 and 15 cm , while the systolic pressure reached 92 With 40 c c diastolic pressures from 17 to 21 were recorded, and pain was now felt throughout The systolic pressure was no higher than before Again, on Jan 3rd, 1922, 60 c c were introduced slowly into a bag of 200 c c capacity and the diastolic pressure rose to 19 cm But when 70 c c were introduced suddenly the pressure rose to 23 cm and pain was felt

There are two possible ways of relieving the stretching and deformity of the pain endings

(1) The circumference—in other words the "posture"—of the organ may be increased by the lengthening or rearranging of all the structures in the wall Exp 8 on E P P is a very good example of this The diastolic pressure with 45 c c gradually introduced into the bag varied between 14 and 26 cm and no pain was experienced This is a great contrast to an experiment with this subject three days later (2nd Exp 25th May, 1922) when with 40 c c the lowest diastolic pressure recorded was 24 cm , and values over 30 were often obtained and pain was felt most of the time The same thing was noticed when the posture varied while the volume of air in the bag was kept constant Thus, in the case of E P P (1st Exp 25th May, 1922) with a bag containing 40 c c of air, and situated 13 inches below the lips no pain was felt with a diastolic pressure of 27 cm , it was felt continuously when the diastolic pressure was 36 cm for a period of 50 sec without any contractions Afterwards the subject vomited up the bag

(2) The muscle may contract This increases the tension of the muscle fibres, but owing to the shortening of the circumference diminishes the stretching and deformity of the pain endings themselves In fact the muscle wall takes the strain from off them It is also easy to imagine an intermediate state when contraction causes diminution in pain

without completely abolishing it An example of this was shown in Exp 2, where pain was continuous, but there were exacerbations between the contractions

However, in the application of our theory some further considerations are necessary

A In Exp 1 with the two bags containing 5 c c and 70 c c the pressure exerted by the œsophageal wall was permanently increased at the site of the two bags, but the actual state of affairs was different in these two places In both the diastolic pressure was increased, but it was much higher at the lower bag The most striking difference was that at the upper bag there was a continuous contraction—a regular tetanus —interrupted at intervals by short deep negative waves due to peristalsis, while at the site of the lower bag, which is to be looked upon as the foreign body causing the disturbance, the pressure remained most of the time at the diastolic value, and a higher systolic pressure was produced only momentarily The pain was produced by the lower bag, but a special strain on the pain endings might be expected to exist at the zone between the muscle in systole above, and the muscle in diastole below In fact "painful spasm" would not be an inappropriate description of this condition in which a peristalsis is held up by an obstruction The strain would be relieved in proportion as the contractions above succeeded in passing down and compressing the lower bag and it would be increased if the contraction above was increased without making any impression on the bag below

There are two pieces of evidence in favour of this view

(1) In the case of a patient described previously (1, Case 1) two pains were felt, one at the top of the sternum, and another at the xyphisternum, while X-rays showed a narrowing at the upper and lower end of the œsophagus with a dilatation between The localisation of the pain at these two points might have been due to the strain on the pain fibres at the zones between the contracted and dilated parts

(2) In Exp 4, when the incompressible bag was filled with water pain was felt at once It was worse during peristaltic contractions, but it only reached its maximal value after a voluntary swallow when a maximal high pressure was produced above the bag by means of a tetanus of many seconds' duration This would lead to the strain already described The filling of the bag in the first place would produce stretching and deformity of the pain endings, but if this was the only factor concerned the pain would have remained constant until the water was let out of the bag, since owing to the incompressibility of the bag the

tension on the pain endings at this point could not have been relieved by muscular contraction This experiment probably has its exact counterpart in the pains of labour, renal calculus and gall stones

B When a pain of short duration was felt it was recorded during the relaxation stage of a contraction (see Fig 5 A), and when the pain was of longer duration it began during the relaxation, which suggests that it is during relaxation that tension is most likely to be exerted on the endings, especially when the fall of pressure is large and rapid W H Ogilvie(25) has made a similar observation on the gall bladder We have already suggested that muscle tonus may act as a kind of catch mechanism which comes into play to counteract a rapid large fall of pressure On the whole it was found much easier to produce severe pain in W W P than in E P P Further, W W P is inclined to suffer from indigestion, while this is not the case with E P P We have seen that high diastolic pressure and high tonus is painful, but it may be less so than the sudden stretch of pain endings which occurs during a large rapid muscular relaxation Is it possible that the difference between W W P and E P P lies in the greater development of the catch mechanism in E P P and that this is associated with a larger diameter of the œsophagus? Carlson(21) has noticed similar individual variations in the stomach

C The character of the pain produced in our experiments varied according as it was continuous or intermittent In the former case it had a burning character, and was reminiscent of heartburn An unpleasant sensation referred to the throat, but also situated deeply at about the level of the suprasternal notch, was present if the bag was high up in the œsophagus If it was lower down the pain was usually referred to the costal angle, and the lower part of the sternum in the mid-line When the pain was intermittent it had what was best described as a gripping character, and the relation of the two types of pain was well seen in an experiment on W W P on May 25th, 1922 (Fig 5 B) At first with 45 c c in the bag continuous severe burning pain was noticed The diastolic pressure was 30 cm , and there were only respiratory variations A swallow was then taken, and this was followed by a series of peristaltic contractions each of which caused diminution in the pain and converted it from a burning pain into a gripping pain Later on the contractions stopped, and the pain again became continuous and burning We noticed the same thing on another occasion when after a period of severe continuous burning pain without contractions, a little air was let out of the bag Large peristaltic contractions then

appeared which made the pain intermittent, and so gave it a gripping character

After a prolonged experiment entailing much pain cutaneous hyperæsthesia was once felt by W W P over the lower part of the sternum, and several times some 10 min to 1 hr after the removal of the bag a pain was felt behind at the angle of the left scapula, as if the muscles were stiff, and there was tenderness on palpation Another subject who kindly volunteered on one occasion felt pain in the back when the bag contained 40 c c Further, we have to record that experiments of this kind may produce unpleasant results subsequently Thus E P P who never has indigestion woke up with quite a severe attack on the day following a painful experiment, and W W P has found that his dyspepsia was made worse by the experiments, and even now more than two and a half years after the last experiment he still fells a little uncomfortable after breakfast We may assume that these experiments caused damage to the structures in the wall. Obviously there is no fundamental distinction between pain produced experimentally and the pain of organic visceral disease

D It would seem that there is quite a close analogy between our present experiments and our previous observations on patients who complained of pain Fig 7 B and c of this paper illustrate the dilatation of the normal œsophagus by means of air bags Pl I of our previous paper presents a very similar appearance of œsophageal dilatation in a patient complaining of heart-burn. It suggests that the œsophagus produces pain by acting as its own air bag Then again, the pain of our present experiments was associated with an increase of diastolic pressure, and this was also the case in the majority of our previous observations on the stomach and small intestine as well as the œsophagus, though the term mean pressure was used for diastolic pressure Finally, in both cases the contractions were usually increased in number as the diastolic pressure rose However, we have now found that when the diastolic pressure or tonus is very high—in other words when the stretch by the bag is very great—no contraction can get past the affected spot and there is continuous and often severe pain This was the condition of affairs in Figs 6, 7 A, and 7 B, c In Fig 5 B it required the stimulus of a swallow to start a series of contractions which could compress the bag, and even these died away after a time It is probable that in our observations on patients the tonus was never so high as to stop contractions altogether, but the observations of Reynolds and McClare[20] that the onset of pain coincided with the dying away of peristalsis can be

explained in this way   However, we did find that diastolic pressures in the stomach associated with pain were usually much below those in the œsophagus   It is clear from the formula $t = pr$, that the larger the posture the less will be the pressure required to produce a given tension, and this may possibly account for the difference between the stomach and œsophagus

The theory of visceral pain which we have described is an extension of that suggested first by Sherrington(26), then by Hurst(5), and more recently by Ryle(18)   A W Mayer(22) has claimed that contracture as well as stretching is a cause of pain in the intestine, and that contracture produced by injecting $BaCl_2$ into the peritoneal cavity produces pain by forming rigid loops of intestine which *stretch the intervening mesentery*   Stretching of the mesentery may possibly be an additional cause of pain where a mesentery exists, though Mackenzie's observation(27) is rather against this, but it is very unlikely to be the chief cause of pain produced by barium   We have poured $BaCl_2$ into the peritoneal cavity of an anæsthetised cat   The effect of the application was to divide the gut sharply into many contracted and dilated portions, and in the latter the contents appeared to be under considerable pressure   It looked as if the activity had caused the contents to be squeezed out of the contracted parts into the intervening dilated portions   In some places rapid peristaltic waves were seen, in others there were none, i e peristalsis was held up in its progress, and this would give rise to severe pain as illustrated in Fig 3

Hurst(23) has recently elaborated his tension theory of visceral pain along rather similar lines to account for hunger pain in the stomach   He suggests that as a peristaltic contraction advances over the pyloric part it causes increased pressure, and so increased tension not in the proximal part of the stomach, but in the wall intervening between the contraction itself and the closed pylorus, and consequently pain is produced here   This mechanism might act if the walls of the stomach were pressed tightly together at the site of the peristaltic contraction as he suggested previously(5), but if a gap was left it would only act if there were lumps of food present or the viscosity of the stomach contents was so high that they could only with difficulty be squeezed back through the gap   But this would seem unlikely in hunger   Further, Reynolds and McClure have found from X-ray examination that peristalsis sometimes ceases at the onset of pain, though it must be admitted that these authors took no account of the possibility that the pain was produced in the œsophagus   We, also, had already published a tracing

(1, Fig 20) which showed that a momentary hunger pain was signalled after contraction of the stomach, and we had found that exactly similar contractions were recorded one second later in the duodenum, which is strongly suggestive of the pylorus being open, so that Hurst's theory could not be applied in this case

Cannon and Washburn in their original communication and Carlson more recently have spoken of hunger *contractions*, though the former stated that they occurred before the sensation of pain Their tracings show both contractions and rise of tonus, but the rate of the drum was so slow that it is difficult to analyse them closely We see no reason why the rise in tonus rather then the contractions should not be held responsible for the pain Further, Carlson has found that hunger sometimes causes a great rise of tonus with contractions of diminishing amplitude which he calls an incomplete tetanus This result is quite like that shown in our lower record in Fig 3 (Exp 1), where the pain was continuous, showing only minor variations in intensity Again, a tracing of A J Carlson's (21, p 70, Fig 12) does suggest to some extent that pain is felt during muscular relaxation of the stomach

Sir James Mackenzie(27) has suggested that contraction of the bowel into a "thick fleshy rod" produces pain But the description of the observation that he gives suggests that he had clamped the gut preparatory to performing an anastomosis and in this case pain might have been caused by the holding up of peristalsis as already described Further, we know that the firmly contracted uterus after parturition is not painful, nor is the firmly contracted colon in muco-membranous colitis

W L Palmer(19) has shown that the hydrochloric acid of the gastric juice may cause pain in patients with gastric ulcer, etc , though not in healthy subjects We certainly agree that in some patients washing out the stomach with dilute hydrochloric acid, or its administration, is painful, but these facts are not inconsistent with the mechanical theory of pain which we have put forward, because the presence of acid may alter the tone of the muscle, and so bring the pain mechanism into action, as he himself is inclined to suggest

CONCLUSIONS

(1) Bayliss and Starling's law of the Intestine or Myenteric Reflex has been demonstrated in the lower end of the œsophagus and applied to œsophageal peristalsis

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Conclusions

(1) Bayliss and Starling's law of the Intestine or Myenteric Reflex has been demonstrated in the lower end of the œsophagus and applied to œsophageal peristalsis

(2) Œsophageal waves resulting from swallowing, secondary swallowing and secondary peristalsis, have been described

(3) Tonus in plain muscle has been defined as the tension of the muscle during diastole Pain produced experimentally is associated with high tonus

(4) As the result of kymographic and radiographic examination of the normal œsophagus it is suggested that visceral pain results from a stretching force in the viscus and is caused by stretching and deformity of the pain endings in the wall It is relieved by a peristaltic contraction that overcomes the stretch, or by postural adaption which increases the capacity of the viscus It is made worse by a peristaltic contraction which is unable to overcome the stretch, and which is consequently held up in its course The latter causes "painful spasm"

(5) "Burning" pain results from a continuous stretching and deformity of pain endings When relieved periodically by successive peristaltic waves it becomes "gripping" in character Pain is especially apt to occur during the muscular relaxation that follows a peristaltic wave Visceral pain experimentally produced if prolonged and severe is followed by cutaneous hyperæsthesia and other signs of referred pain, and may produce unpleasant results later

(6) The application of these findings to previous results obtained by others and ourselves is considered

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THE ACTION OF INSULIN ON THE ASEPTICALLY PERFUSED HEART

BY R BODO AND H P MARKS

(From the National Institute for Medical Research, London)

Cousy(1), of Noyon's laboratory, has recently repeated the perfusion experiments of Hepburn and Latchford(2) on the isolated rabbit's heart, in which they observed an increase in apparent sugar disappearance following the administration of insulin   But, whereas under ordinary conditions, the former observes a similar increase, when aseptic precautions are taken, he finds the sugar consumption to be practically abolished, instead of increased Thus, in a number of experiments performed in the ordinary way, the apparent sugar disappearance was increased on the average from 1 08 to 1 85 mgr per gr of heart per hour under the action of insulin, while in similar aseptic experiments it was reduced from 0 85 to 0 15 mgr per gr per hour

From these results Cousy concludes that insulin inhibits the glycolytic action of the heart, and attributes the increase in sugar disappearance normally observed after insulin to the action of bacterial contamination Since this explanation appeared highly improbable in the light of previous work on perfused heart and skeletal muscle, carried out in this Institute by Burn and Dale(3), it seemed desirable to repeat Cousy's aseptic experiments

Our first difficulty arose in connection with the preparation of sterile Ringer-Locke solution   Unfortunately, Cousy does not give any details concerning his perfusion solution, except that he boiled his solution and brought it back to the original volume   He does not mention the composition of the solution which he boiled, although it is well-known that this procedure leads to loss of carbon dioxide and precipitation of the calcium carbonate   We were only able to avoid this by sterilising the sodium bicarbonate solution separately from the other salts

The procedure was as follows The bicarbonate solution, to which was added a drop of phenolphthalein, was first boiled and cooled, during which process it became carbonate through loss of carbon dioxide This latter was replaced by leading in a stream of the gas until the deep red

colour was discharged, and then the excess of carbon dioxide was removed by oxygenating the solution until the original faint pink colour appeared. The bicarbonate solution was then mixed into the solution of the remaining Ringer salts, previously boiled cooled and thoroughly oxygenated, and, after addition of the glucose solution (sterilised three times in steam) was finally made up to volume in a sterilised one litre flask

Aseptic precautions were observed throughout The necks of all flasks were protected from contamination by inverted beakers, and were flamed before the transfer of solution The tube for leading in carbon dioxide and oxygen was provided with a bulb containing a plug of sterile wool, and with a glass hood to protect the neck of the flask and was previously sterilised in the autoclave (The effectiveness of these precautions was always controlled by preparing agar plates from the final solution) The solution so obtained was identical in its composition with that described by Locke and Rosenheim(5), and had the same pH, but was also sterile

All flasks were of either Jena or Pyrex glass, and the distilled water used was previously redistilled in Pyrex glass apparatus in order to eliminate traces of heavy metals The salts used were Kahlbaum s puriss and the composition of the solution was as follows

NaCl 0 9 p c   KCl 0 042 p c , CaCl$_2$ anhydr 0 024 p c , NaHCO$_3$ 0 02 p c , Dextrose 0 1 p c

In one or two experiments a borate Ringer of the following composition was employed

NaCl 0 9 p c   KCl 0 042 p c , CaCl$_2$ anhydr 0 024 p c , Dextrose 0 1 p c , 20 c c Palitzsch's(4) borax-boric acid solution of pH 7 6 (3 c c M/20 borax − 17 c c M/5 boric acid M/20 NaCl)

The preparation of this solution was quite simple, since it could be sterilised by boiling without fear of decomposition but we preferred to use in most cases the more physiological Ringer-Locke solution

The sterilisation of the apparatus did not present much difficulty A suitable form of apparatus has been described by Novons(6), and this was employed by Cousy We used a closed circuit perfusion system similar in principle but modified so as to permit easier sterilisation and aseptic manipulation By keeping as much of the apparatus as possible immersed in warm water jackets, it was found possible to dispense with the spiral heating tube employed by Novons, and consequently to reduce the volume of the apparatus so that a perfusion could be performed with as little as 25 c c of fluid Means were provided for the addition of fluid

to, or removal from the apparatus under aseptic conditions, through the graduated tap funnel (*a*) and the shielded jet (*b*) respectively The apparatus was maintained at a temperature of 39° C by means of a current of water heated by the electric immersion heater (*h*) and flowing first through the upper, and then through the lower water jacket, as indicated by the arrows in the diagram A prolonged contact of the water with the heater was ensured by surrounding the latter with a copper wire spiral The temperature of the water was regulated by adjusting the rate of flow past the heating element, this being done by raising or lowering the overflow tube (*e*)

The apparatus was sterilised (in steam) in three portions, viz the part contained in the upper jacket, the heart chamber, and the heart cannula attached to a temporary reservoir by a length of rubber tubing

The upper part of the apparatus was first inserted into its water jacket and the current of heating water adjusted to give a temperature of 39° C in the jacket The upper reservoir (*f*) and the temporary reservoir, with attached heart cannula, were then filled with the warm sterile Ringer's solution

The actual experiment was performed under strict aseptic precautions, the rabbit's breast and neck having been depilated and painted with iodine Under ether

Fig 1.

anæsthesia we bled the animal from the carotid artery, and, after opening the chest, the sterile cannula, connected with the temporary reservoir, was inserted and tied into the aorta The heart was isolated and perfused in a sterile dish until the outcoming fluid contained no more blood The temporary reservoir was disconnected and the cannula attached to the apparatus by the junction (*j*), the perfusion being continued from the upper reservoir, until the level of the Ringer had fallen to a mark which indicated that the amount of fluid left in the apparatus was exactly 10 c c The perfusion was then momentarily stopped while the heart chamber was attached, and 52 5 c c of fresh Ringer run in from the tap funnel (*a*) After perfusing the heart, the Ringer's solution drains from the heart chamber and is carried by a stream of oxygen issuing from the jet (*g*) back to the upper reservoir, from which it again passes to the heart through a vertical drop of about 60 cm The mixed fluid and oxygen enter the upper reservoir through the greased spiral of silver wire (*w*) which effectively breaks up the froth, while the glass-wool plug (*p*) prevents any solid particles from reaching the heart and causing an embolism

The oxygen enters the apparatus through the gas-washer (*d*) and the sterile wool plug (*i*), and leaves by the sterile trap (*c*) That the loss of fluid by evaporation is insignificant is shown by the fact that the theoretical amount of fluid can be recovered from the apparatus after a perfusion lasting for four or five hours

About ten minutes after the commencement of the perfusion proper, samples of fluid for bacteriological control and determination of sugar were removed by means of a sterile syringe and needle inserted through the rubber junction (*j*)

Initially 12½ c c of fluid were removed, two portions of 5 c c being mixed with glucose-agar and poured into Petri dishes for incubation at 37°C , and 2 c c being deproteinised with zinc hydroxide for sugar determination, by the Hagedorn–Jennsen method

Further samples were removed for sugar determination at half-hourly intervals, and samples for bacteriological control in addition, at the end of each perfusion period After a control period of one hour, as much as possible of the perfusion fluid was removed by the jet (*b*) and replaced by an appropriate amount of fresh Ringer containing the required amount of insulin By this means metabolic products were removed as far as possible, and the glucose concentration brought back nearly to the original value, so as to have the same conditions in the second period as in the first After five minutes' perfusion to allow thorough mixing,

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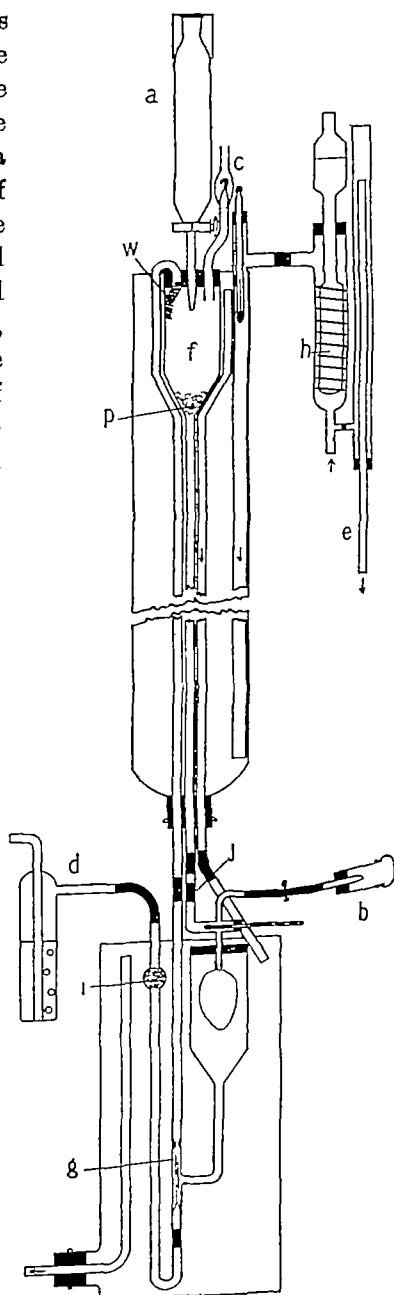

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ınıtıal samples for the period were withdrawn   After one hour ınsulın period, the process was repeated for a second ınsulın period where possıble

The followıng ıs a typıcal experıment ın full

TABLE I.

Time		Sugar con sumptıon ın mgr per gr per hour	Tempera ture °C	Heart-rate per mın.	Outflow ın drops per mın.	Bacterıal contamı natıon per c.c.
Control perıod						
1 5	1st sample	0 6	39 0	165	165	0
1 35	2nd sample	1 0	39 0	174	171	—
2 5	3rd sample		39 2	171	168	0
1st Insulın perıod (1 unıt)						
2 25	4th sample	2 9	39 2	174	165	—
2 55	5th sample	2 0	39 0	174	156	—
3 25	6th sample		39 0	174	138	0
2nd Insulın perıod (1 unıt)						
3 37	7th sample	1 8	39 0	153	126	—
4 7	8th sample	2 0	39 0	153	100	—
4 37	9th sample		—	—	—	8

One unıt of ınsulın was used ın all such experıments Thıs amount ıs sufficıent to produce a well-marked effect ın the whole anımal and should therefore suffice for the ısolated heart   We dıd not use the large doses employed by Cousy, (20–40 unıts) as one of us (R B) has found that, ın the heart-lung preparatıon, even of the dog, such doses produce a heart dılatatıon and constrıctıon of the coronary vessels On the other hand, one unıt has no constrıctor effect on the coronary vessels The experı ments were consıdered satısfactory only so long as the temperature, heart-rate, and coronary flow (measured by the drops fallıng from the heart apex) remaıned practıcally constant Sometımes ın the later perıods the coronary flow would change, through capıllary embolus or capıllary œdema, and such perıods are not ıncluded ın the table sum marısıng our experıments

In order to observe the effect of the procedure of changıng the Rınger on the apparent sugar dısappearance, a few control experıments were carrıed out ın the same way, but wıthout the addıtıon of ınsulın to the fresh Rınger used ın the later perıods The followıng control experıment (Table II) ıs typıcal

From Table III, whıch summarıses our results, ıt ıs seen that ınsulın ıncreases the apparent sugar dısappearance ın our aseptıc ex perıments at least as much as ın the non-sterıle experıments of prevıous workers

TABLE II.

Time	Sugar consumption in mgr per gr per hour	Temperature °C	Heart-rate per min.	Outflow in drops per min.	Bacterial contamination per c c
First period					
1 50 1st sample	0 5	39 2	180	180	3
2 20 2nd sample	1 7	39 2	180	180	—
2 50 3rd sample		39 0	180	180	3
Second period					
3 8 4th sample	1 4	39 0	180	180	—
3 38 5th sample	1 1	39 2	180	180	—
4 8 6th sample		—	—	—	1
Third period					
4 20 7th sample	1 0	39 2	180	162	—
4 50 8th sample	0 8	39 0	165	102	—
5 20 9th sample		39 0	180	90	5

TABLE III.

| Expt. No | Sugar consumption for each half hour in mgr gr hr | | | | | | Bact contamination per c c | |
	1st hour control		2nd hour insulin		3rd hour insulin		Initial	Final
1	1 0	1 0	2 0	1 2	1 4	—	0	5
2	1 0	1 0	3 2	2 0	1 8	1 9	1 4	0 9
3	0 6	1 0	2 9	2 0	1 8	—	0	0 8
	Control		Control		Control			
4	0 5	1 7	1 4	1 1	1 0	—	0 3	0 5
5	1 6	1 2	1 5	1 1	—	—	0 6	1 2
6*	1 4	0 8	1 2	1 3	2 2	1 8	—	—
7*	1 1	1 3	1 1	1 1	1 4	—	40	120

* Without aseptic precautions.

It will be seen from Table III that we did not achieve absolute sterility of working in these experiments We doubt, indeed, the possibility of carrying out the relatively complicated technique of this perfusion in such a way as to exclude a very small number of bacteria with complete regularity We took the precaution, however, of making regular bacterial counts from samples of the fluid taken at intervals, the results of these, as shown in the tables, make it clear that the contamination was minimal, and that no serious multiplication took place during the course of the experiments Certainly there was no such growth in the experiments aiming at asepsis, as could account for any significant part of the large acceleration of sugar disappearance which followed the addition of insulin, nor was there any evidence that the addition of insulin influenced the rate of bacterial multiplication In Cousy's paper, where the effect of insulin is apparently attributed to bacterial growth, there is no record of bacterial counts, or any other indication that direct evidence was

obtained of a connection between insulin and bacterial action In two control experiments, without insulin or asepsis, we observed an apparent increase of sugar consumption in the final period, which could reasonably be attributed to the bacterial growth, then probably becoming active, but this could have no connection with the effect seen in the insulin experiments, where a much larger acceleration of sugar disappearance followed the introduction of insulin without any effect on the practically aseptic conditions

CONCLUSION

From the above results we draw only one conclusion, namely that the accelerated disappearance of sugar, from the fluid perfusing an isolated mammalian heart, under the influence of insulin, is a real phenomenon, and is not connected with bacterial contamination of the perfusion fluid Our experiments throw no new light on the question of the fate of the sugar so disappearing They show, as did those of Burn and Dale, that a large acceleration of sugar disappearance may occur without any sig nificant acceleration of the heart beat From analogy with the effect of insulin on skeletal muscle it would be expected that part of the glucose so disappearing is stored as glycogen, but it is not possible to carry out on the heart such adequate control analyses as would enable such a storage to be demonstrated

SUMMARY

1 Apparatus and technique for aseptic perfusion of the isolated heart are described

2 The increase in sugar disappearance after insulin, observed by previous workers, also occurs under sterile conditions

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THE PERMEABILITY AND DIAMETER OF THE CAPILLARIES IN THE WEB OF THE BROWN FROG (*R TEMPORARIA*) WHEN PERFUSED WITH SOLUTIONS CONTAINING PITUITARY EXTRACT AND HORSE SERUM By CECIL K DRINKER

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In 1921 Krogh and Harrop[1] perfused the leg of the brown frog through the femoral artery and found that the addition of defibrinated ox blood to a 3 p c acacia-Ringer solution had a pronounced effect in preventing capillary dilatation This effect, though somewhat variable in degree, was sufficiently vivid to cause the authors to prepare dialysates of blood serum, which, when added to Ringer's solution plus washed ox red cells or to 3 p c acacia-Ringer's solution plus washed ox red cells, also had an effect in preventing capillary dilation, or, in the usual phraseology, in maintaining capillary tonus If unsuitable perfusion fluids were used, the appearance of œdema was noted in these experiments but, since at that time the actual diameter of the capillaries was the subject under observation, the possible relations between dilatation and appearance of œdema were not followed Later experiments, into the details of which it is unnecessary to go, suggested that secretion from the posterior lobe of the pituitary gland might well be the ultimate constituent of serum which maintained the capillaries at normal diameter

During the past six years, experimental attention has centred more upon the action of histamine and histamine-like substances which cause capillary dilatation and œdema, than upon the possible existence of a substance which maintains the capillaries in that normal state of contraction we call tone In relation, however, to the position of pituitrin as the agent for this important end, several points have been made Carrier[2] found that pituitrin (Parke, Davis & Co) in strong concentrations, introduced around the capillaries at the base of the nail, caused them to constrict Kolls and Geiling[3] obtained marked capillary constriction in the dog's ear after giving large intravenous injections of pituitary liquid Sacks[4] obtained a similar result in man and with more dilute solutions Coincident with capillary constriction, Sacks was able to show a restraint of œdema formation Krogh[5], in 1926, utilising

melanophore dilatation as a means of detecting the presence of the pituitary hormone in perfusion experiments on the legs of brown frogs, found it possible to show the presence of traces of this substance in normal horse serum These observations caused him to suggest again that one of the functions of pituitary secretion may be the maintenance of capillary tone   Dale(6) has recently summarised the evidence which has led him to the conclusion that pituitrin alone is not an important agent for this end

In summary, then, it has been found that serum possesses the power to prevent capillary dilatation, that pituitary extracts cause contraction in frog and mammalian capillaries, and that traces of pituitrin, frog melanophores being used as indicators, exist in mammalian serum The experiments which follow constitute an attempt to devise a preparation which will permit photographic records of the web capillaries at repeated intervals during long experiments, combined with accurate measurements of perfusion flow and onset of œdema The results obtained in the case of pituitary extract are not in conformity with the hypothesis that pituitrin is an effective agent for maintenance of capillary tone, but, as will be pointed out, this contradiction has appeared as the result of an experimental procedure not entirely similar to that originally used by Krogh and Harrop(1), and serves more as an indication of the complicated nature of the action of pituitrin than as a certain negation of pituitary influence upon tone

TECHNIQUE

A Perfusion apparatus The perfusion apparatus is essentially that described by Krogh(1), namely, a rhythmically operative air blast playing upon the surface of the perfusion fluids and forcing them towards the animal Systolic and diastolic pressures are quickly adjustable and are reasonably constant Since the fluids used have contained no corpuscles, the mixing reservoirs employed by Krogh have been supplanted by 10 c c graduated pipettes Each is arranged as shown in Fig 1 A row of these pipettes in a stand permits the use of different fluids in an experiment. As delivery progresses, the pressure obviously falls This does not, however, affect the accuracy of the figures for blood flow since these invariably cover given periods of time, usually ten minutes, at the end of which the fluid is again drawn up to a uniform starting level As a consequence, pressures during each period of perfusion are the same

B Preparation and arrangement of frog For perfusion purposes, the arrangement of the vessels in the leg and foot of the frog are singularly

advantageous The anterior tibial artery is easily found under the external margin of the tibia, just above the ankle joint It passes under the cruro-tarsal ligament and becomes the dorsalis pedis, the principal source of blood supply for the dorsum of the foot Both dorsal and plantar blood vessels anastomose freely and unite in supplying the web, the dorsal supply being somewhat the larger Anastomosis also occurs proximal to the web and one can readily develop situations in which fluid, introduced through the anterior tibial, passes by these connections into the plantar vessels and flows out of cut arterial twigs without entering the web capillaries The web cannot be perfused thoroughly if other arteries are not completely obstructed and the venous outflow free

To accomplish these results, the following dissection is made The skin of the leg is slit on both internal and external surfaces from the ankle to the knee, and the resulting anterior and posterior ribbons of skin are cut between ligatures These ligatures are important not only for obstructing cut skin vessels but even more because they draw the skin tight around the ankle and prevent escape of fluid if œdema occurs The gastrocnemius tendon is freed and cut between ligatures The muscle is then loosened up

Fig 1 Perfusion burette. Reservoir 1 contains perfusion fluid to be drawn into the burette by suction on Tube 3 Tube 2 being open and Tubes 4 and 5 shut Air blast through Tube 4 and fluid delivery through Tube 5

to its origin, care being taken to ligate all blood vessels It is imperative that the peroneal vein, which becomes the popliteal behind the knee joint, should be the only possible point of escape for the perfusing fluid When the gastrocnemius is freed to the knee and is drawn back, the popliteal vein is fully displayed and is readily cut after the perfusion has been started. The sciatic nerve is now freed just above the knee and a heavy ligature passed under the nerve and around the thigh. When this ligature is tied, the leg is isolated as far as circulation is concerned, but the nervous control of the vessels remains

At this point in the dissection, the frog is placed, back down, upon a

brass tray (1, Fig 2) This tray can be attached to the movable stage
of the microscope by a turn of the long screw, 2, and by the lugs, 3 and 4
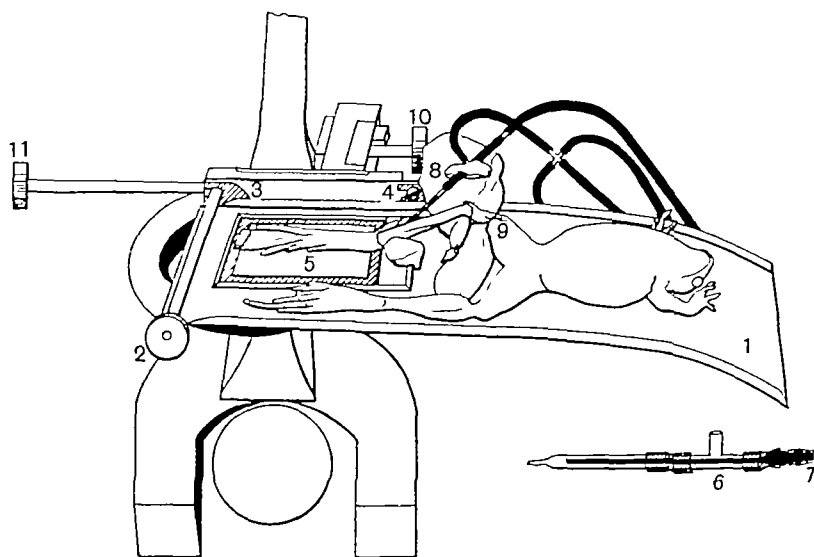
Fig 2   Arrangement of the frog for perfusion of the web and micro photography
of the capillaries

It possesses a window, 5, closed by a movable plate of glass The frog's
foot rests flat upon this plate, the position being readily obtained by
bending the knee over a lump of plasticine With the frog arranged upon
the brass tray, which is not as yet fastened upon the microscopic stage,
the anterior tibial artery is exposed just above the ankle joint, and two
ligatures of fine silk are placed under it In 50 gm frogs the vessel is
about 0 2 mm in diameter Its dissection, therefore, and the subsequent
cannulation require the use of the binocular microscope The cannulas
used are best made of clear quartz tubing Pyrex tubing is somewhat
easier to work, but I have been unable to make from it cannulas which
possess at the tip an outside diameter of not more than 0 4 mm in the
bulging part and which still retain a good-sized opening The toughness
of clear quartz permits one to make cannulas which are strong enough to
withstand vigorous tying and which, at the same time, possess an ex-
tremely thin wall and a consequently relatively large lumen It is
necessary that the cannula tip be evenly bevelled by grinding on a razor
hone under the microscope and that the point be smoothed by flaming
    The cannula is connected with the delivery tubing from the pressure

burettes by means of a "T," as shown in 6 (Fig 2) This is done to permit the insertion of a capillary tube which is connected by tubing at 7 with the pressure reservoir containing graphite ink The arrangement is exactly that of the Brodie washout cannula and permits the shift from clear perfusion fluid to graphite ink without delay The cannula and tubing having been filled with perfusion fluid and with ink, the proximal ligature upon the anterior tibial artery is tied and the vessel is opened by grasping the wall with finely pointed forceps and making a cut with forceps-scissors of the type described by Krogh(5) The cannula is introduced and is then held in place by embedding it in a lump of plasticine (8, Fig 2) and by covering it with a small slip of the same substance The perfusion is then started With the fluid running the distal ligature is tied securely around the neck of the cannula and the cannula is adjusted so as to lie in perfect alignment The ligature, 9, about the thigh is now tied and the popliteal vein cut behind the knee The brass tray carrying the frog, with the perfusion fluid running, is next moved to the stage of the microscope and locked there by means of the screw, 2, and the two lugs, 3 and 4 The adjusting screws, 10 and 11, of the movable stage readily bring the frog's web into the line of microscopic vision

The arrangement described results in the perfusion of the skin of the foot, together with the muscles and bones The leg muscles receive slight but variable amounts of perfusate on account of the anastomotic loops with the plantar vessels, and a very minor fraction may even reach the gastrocnemius The effluent fluid comes from the popliteal vein alone

The time which elapses between the tying of the anterior tibial artery and the introduction of the cannula and the starting of the perfusion is ordinarily about two minutes but if it is longer no harm is done, since the plantar circulation continues uninterruptedly until the thigh ligature is tied

Frogs have been curarised and the brain destroyed, care being taken to keep well above the medulla The curare used is one of a number of specimens obtained from South America several years ago and shown in a large number of mammalian experiments to be without appreciable effect on the vascular apparatus Curarisation is necessary since the foot must not move through long periods of microscopic observation during which the same field is photographed again and again Destruction of the brain and cord, while giving a quiescent animal, introduces conditions of vascular dilatation so variable and so poorly understood as to make it impossible to secure preparations which behave uniformly

The perfusion pressures utilised are recorded with the experiments

Landis(7) gives a series of measurements of pressure in the mesenteric arteries of frogs which fall between systolic values of 50 and 24 and corre sponding diastolic values of 24 and 3 cm of water My measurements of mean pressure in the anterior tibial artery of decerebrated, curarised frogs are in the same range Different rates of fluid flow have been em ployed in the various experiments reported in this paper, adjustments being made in accordance with the requirements of the particular experiment

   C Methods of observation and recording A Leitz microscope equipped with the Siedentopf photographic eyepiece (Zeiss) has been employed This eyepiece permits the use of 4 5 × 6 cm. film packs and is ideally adapted for capillary work since one can see the field while photo-graphing

   In the enlarged photomicrographs shown in Figs 5 and 8 *A*, *B* and *C*, it is possible to find capillaries containing graphite ink and, outside the solid band of ink, a fine line which looks like the capillary wall Such appearances express defects in the technique of photographic recording The ink distributes itself evenly in the individual capillaries practically as soon as it enters them and the outer margins of the bands of ink which appear in the illustrations constitute a true measure of capillary diameter

   The charts of individual experiments contain three curves The first of these consists of a series of arrows, each of which gives the average blood flow in cubic centimetres per minute for a period of minutes, covered by the length of the arrow The second curve, a broken line, gives the time in seconds required for the graphite ink to reach the web capillaries after being turned on in the perfusing cannula This curve of ink appearance time is of great importance in indicating the uniform condition of the preparation The third curve is a solid line and indicates the appearance and development of œdema A number of methods have been tried in order to get the earliest possible indication of capillary leakage A capillary manometer has been introduced under the skin, the whole foot has been placed in a plethysmograph, and repeated photographs of the foot have been made Photographs are of great use but, even when measured most carefully, are not ordinarily so sensitive as the method upon which this third curve is based When the final microscopic field is selected, the micrometer on the fine adjustment of the microscope is set at zero and a clear focus on the upper surface of the web obtained with the coarse adjustment If the web swells, this focus hone ___ ___ ʾns must be drawn up by the fine adjustment Con-
   The cannula is conf

stant readings of the fine adjustment micrometer will thus give an indication of swelling or of shrinking—an index which is at its best in the first stages of œdema When œdema has progressed, the web is often lifted or distorted, owing to swelling of the toes A pronounced alteration of focus is, however, in my experience, the best means available for the recognition of early œdema The measurement is, necessarily, not an exact one and no attempt has been made to plot the readings in terms of actual swelling of the web When below the abscissa, the solid-line curve indicates shrinkage, when above, swelling Under the magnification used, differences of twenty points above or below are of no significance A large movement in a single direction indicates a positive change in the foot

D Perfusion fluids A description of the method of preparation and an account of the properties of the graphite ink which is alternated with the perfusion fluids is to be found in a separate paper (6) In the experiments here recorded, the ink has never been allowed to flow continuously for long periods but has been used in order to make possible photographing of the web capillaries and in order to give evidence, at suitable intervals, of the condition of capillary flow

Acacia—obtained from a local firm—has served as a basis for the perfusion fluids A sample specimen of the material used throughout the entire group of experiments was found on analysis to contain

Calcium as CaO   0 80 p c
Potassium as K_2O 0 78 „
Magnesium as MgO 0 32 „

A representative frog Ringer's solution (9) contains 0 012 p c calcium chloride and 0 014 p c potassium chloride If one calculates the calcium and the potassium as chlorides in the above acacia, one will find that in a 3 p c acacia solution—the usual strength employed—there is 0 048 p c calcium chloride and 0 037 p c potassium chloride These figures are high, but since the degree to which the calcium and the potassium of acacia exist in ionised form is undoubtedly low, it is probable that the calcium and the potassium content of the acacia used are reasonably adequate

The colloid osmotic pressure of 3 p c acacia plus 0 65 p c sodium chloride has been measured a number of times (10), and has been found to vary between 72 5 and 78 mm of water The average of many determinations of the colloid osmotic pressure of frog's blood, made with the same membrane, has been 71 mm of water

than has been thought They leak protein-containing fluid at all diameters but this leakage is never excessive unless capillary integrity is lost It is of interest that completely free transudation is accompanied by extreme dilatation, so that one may consider true œdema and large capillary dilatation as inevitably concomitant phenomena

In the following experiments, when capillary œdema is described as occurring, a rapid and large swelling of the foot is meant Under normal conditions of pressure, this type of swelling does not take place unless very hypotonic solutions are used For example, 40 p c horse serum in 0 65 p c sodium chloride has an osmotic pressure identical with frog serum If such a solution is perfused at an excessive pressure, there will ensue a slow swelling of the foot, a swelling which eventually ceases If now the perfusate is changed to a 3 p c acacia in 0 65 p c sodium chloride and the pressure greatly reduced, the foot will again begin to swell and will become hugely distorted if the perfusion is continued The problem which concerns us is the condition of the capillaries when this true œdema occurs and the nature of solutions that will prevent it

The perfusates upon which this paper is based are listed in Table I

TABLE I. LIST OF SOLUTIONS USED IN THE PERFUSIONS

(Osmotic pressures are for the colloids of the various perfusates and have been determined, in all cases, by use of membrane 4 B (see p 255) With the same membrane the colloid osmotic pressure of frog serum averages 71 mm of H_2O The pH of all solutions is between 7 3 and 8)

1 Three p c acacia in 0 65 p c NaCl, with and without pituitary extract Colloid osmotic pressure 75 2 mm H_2O

2 Six p c acacia in 0 65 p c NaCl with and without pituitary extract Colloid osmotic pressure, 211 mm H_2O

3 Three p c acacia plus 5 p c stock horse serum in 0 65 p c NaCl, with and without pituitary extract Colloid osmotic pressure 78 mm H O

4 Three p c acacia plus 10 p c stock horse serum in 0 65 p c NaCl with and without pituitary extract Colloid osmotic pressure 103 mm H_2O

5 Three p c acacia plus 15 p c stock horse serum in 0 65 p c NaCl, with and without pituitary extract Colloid osmotic pressure 111 mm H_2O

6 Three p c. acacia plus 20 p c stock horse serum in 0 65 p c NaCl, with and without pituitary extract Colloid osmotic pressure 126 mm H_2O

Protocols of experiments with 3 and 6 p c acacia, with and without pituitary extract, will not be given Even with perfusion pressures far below those normally present in the anterior tibial artery, these solutions cause the appearance of a true œdema This œdema comes after a latent period of ten to twenty minutes and, once visible, seems to progress rapidly It is as though the capillary walls, washed free of frog plasma,

become completely permeable to acacia Dilatation accompanies this free leakage Acacia in 3 p c solution in physiological saline and properly buffered has a viscosity above frog serum and an identical colloid osmotic pressure when measured with the same membrane But *the capillaries are wholly permeable to it* and pituitary extract added by *itself does* not better the situation It seems, therefore, that any reputation held by acacia as a useful injection material must depend more upon the company in which it finds itself when placed in the mammalian circulation than upon any significant suitability inherent in the gum

One encounters a very different state of affairs when one perfuses with acacia solutions to which stock or fresh horse serum has been added As the concentration of the serum is increased, a point is reached at about
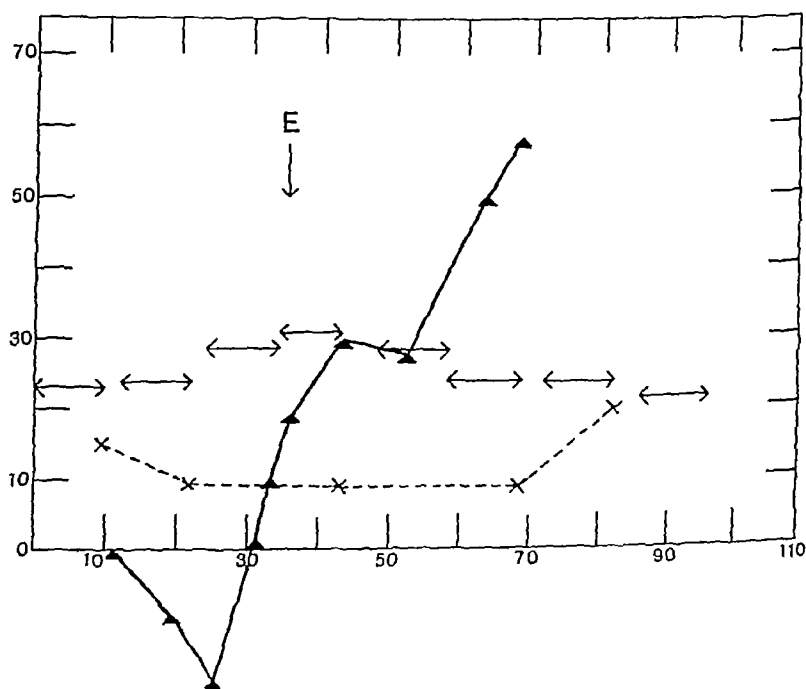
Fig 3 Perfusion with 3 p c acacia plus 5 p c stock horse serum in 0 65 p c NaCl and pituitary extract (200 parts of standard solution in 1 000,000 of perfusing fluid) Abscissa, time in minutes Ordinates c c./10 for rates of flow seconds for graphite ink appearance time, micrometer units for curve showing appearance of œdema Arrows give average rate of flow per min in c.c /10 for a period of minutes covered by their length broken line indicates ink appearance time and solid line, micrometer readings upward movement indicating swelling of the web At *E* œdema was recognisable grossly Pressure, 33–38 5 cm H$_2$O

15 p c where true œdema seldom occurs A series of figures will serve for illustration

Fig 3 is a record of a perfusion with 3 p c acacia plus 5 p c horse serum in the concentration of 200 parts to 1,000,000 of solution Œdema begins in twenty-eight minutes and becomes extreme There is a slight increase in perfusion flow which appears prior to the actual onset of œdema Photographs, similar to those illustrating subsequent experiments, show capillary dilatation, coupled with marked expansion of melanophores at the end of the twenty-third minute of perfusion The addition of serum in 5 p c concentration to acacia is not sufficient to prevent true œdema or capillary dilatation, and pituitary extract does not better the situation

Fig 4 is the record of an experiment of similar type with the horse
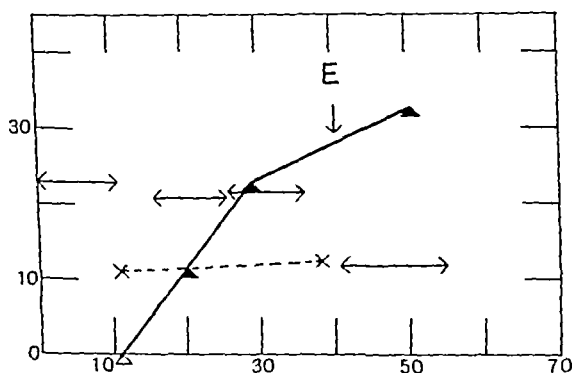
Fig 4 Perfusion with 3 p c. acacia plus 10 p c stock horse serum in 0 65 p c NaCl and pituitary extract (200 parts of standard solution in 1 000 000 of perfusing fluid) Charting similar to that in Fig 3 Pressure 32 4–35 cm H_2O

serum increased to 10 p c Œdema begins early and is steadily progressive The photographic records obtained in this experiment are shown in Fig 5, each enlargement representing a portion only of the microscopic field Picture 1 was taken during the first ink injection after thirteen minutes of perfusion The melanophores are seen just beginning to expand as a result of the pituitary extract in the perfusate Picture 2, taken at the twenty-first minute of perfusion shows the field after the graphite ink has been washed out by the clear perfusate Picture 3 shows the melanophores eleven minutes later Picture 4 shows the dilated condition of the capillaries at the thirty-ninth minute of perfusion when the graphite ink is again flowing under a pressure identical with that operating when Picture 1 was taken In this case, as in the

first experiment, œdema has appeared promptly and has become extreme, and this in the presence of sufficient pituitary extract to cause
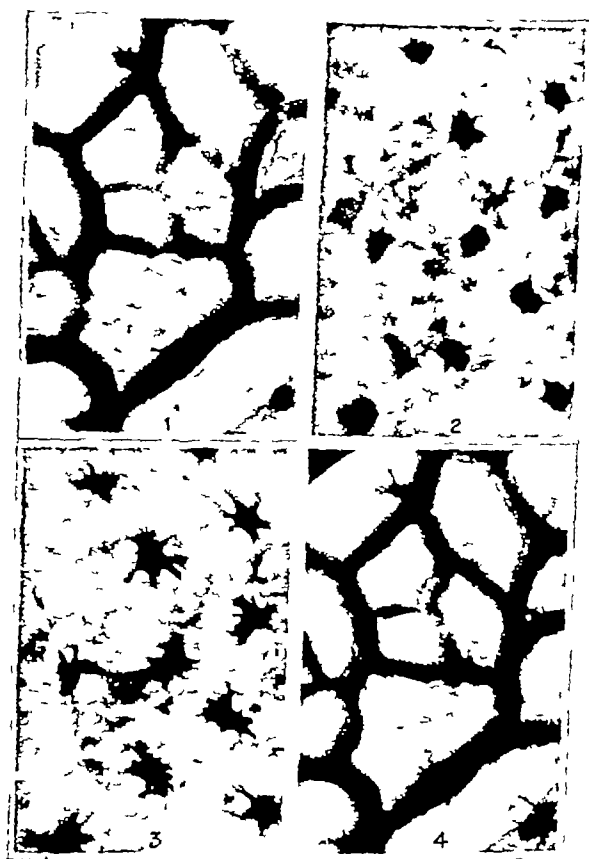
Fig 5   Photomicrographs of a section of the field from the experiment charted in Fig 4.
Magnification × 55

Picture 1   Capillaries made visible by graphite ink after thirteen minutes perfusion.

Picture 2   The same field after washing out the graphite ink with the clear perfusate
Taken after twenty one minutes perfusion   Expansion of the melanophores has begun

Picture 3   The same field eleven minutes later, showing the expansion of the melanophores
which has occurred as œdema has progressed.

Picture 4.   A second perfusion with graphite ink made after thirty nine minutes of perfusion.   Pressure conditions are identical with those in Picture I, melanophores are
markedly dilated, and capillary dilatation is evident

pronounced melanophore expansion   Serum in 10 p c concentration
has never served to prevent true œdema

Fig 6 shows a long experiment in which 15 p c horse serum in 3 p c acacia serves as the perfusing fluid Œdema does not occur during a period of a little over three hours The solid line denoting focal length moves upward to a slight degree in the early part of the experiment, but this upward movement does not continue and any fluid escape from the capillaries which may be taking place is not in amounts above normal During this experiment, œdema appreciable to the eye is at no time evident Perfusion begins with a fluid containing pituitary extract $(F\,1)$ After fifty minutes, a shift occurs to one without pituitary extract $(F\,2)$, which is continued for an hour, then comes a return to the original perfusate, a shift back to the pituitary-free perfusate, and so on to the end Photographs taken during this experiment show the melanophores expanding and contracting in accordance with the presence or absence of pituitary in the perfusates and capillaries which are uniformly of small diameter This favourable result does not constantly occur with 15 p c horse serum in 3 p c acacia since this concentration of serum is just on the edge of effectiveness

Fig 7 shows a long experiment in which perfusion begins with 20 p c horse serum in 3 p c acacia plus pituitary extract Conditions remain very uniform for sixty-five minutes

Fig 6 Perfusion with 3 p c acacia plus 15 p c stock horse serum in 0 05 p c NaCl $F\,1$ is perfusing fluid containing pituitary extract (200 parts of standard solution in 1,000,000 of perfusing fluid) $F\,2$ is the same fluid without pituitary Perfusion begins with $F\,1$ and continues with alternations of the two solutions Œdema does not occur Charting similar to that in Fig 3 Pressure, 27–32 cm H_2O

Fig 8, A, B and C, gives photomicrographs of a single field taken at intervals during the course of an experiment Picture 1, A, was taken
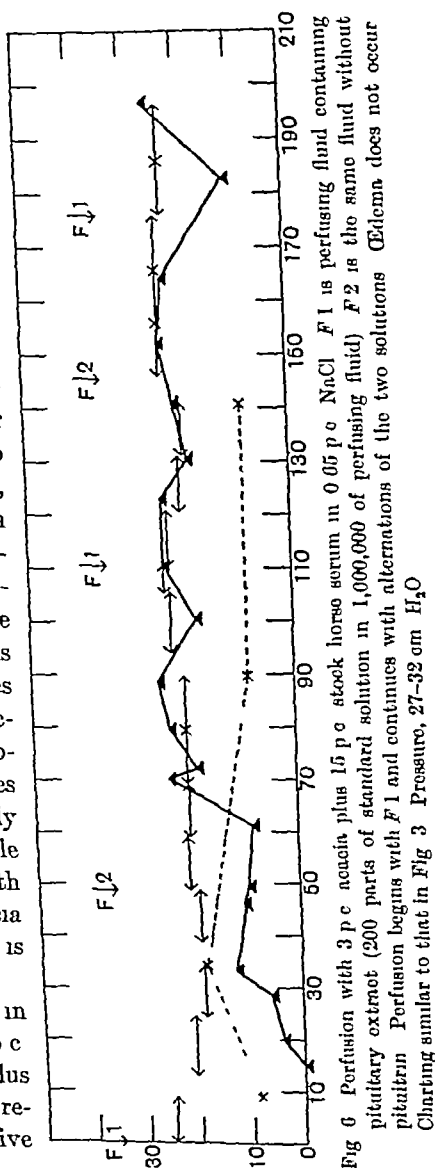
after eleven minutes of perfusion and with a relatively low blood flow It shows narrow capillaries and contracted melanophores Picture 2
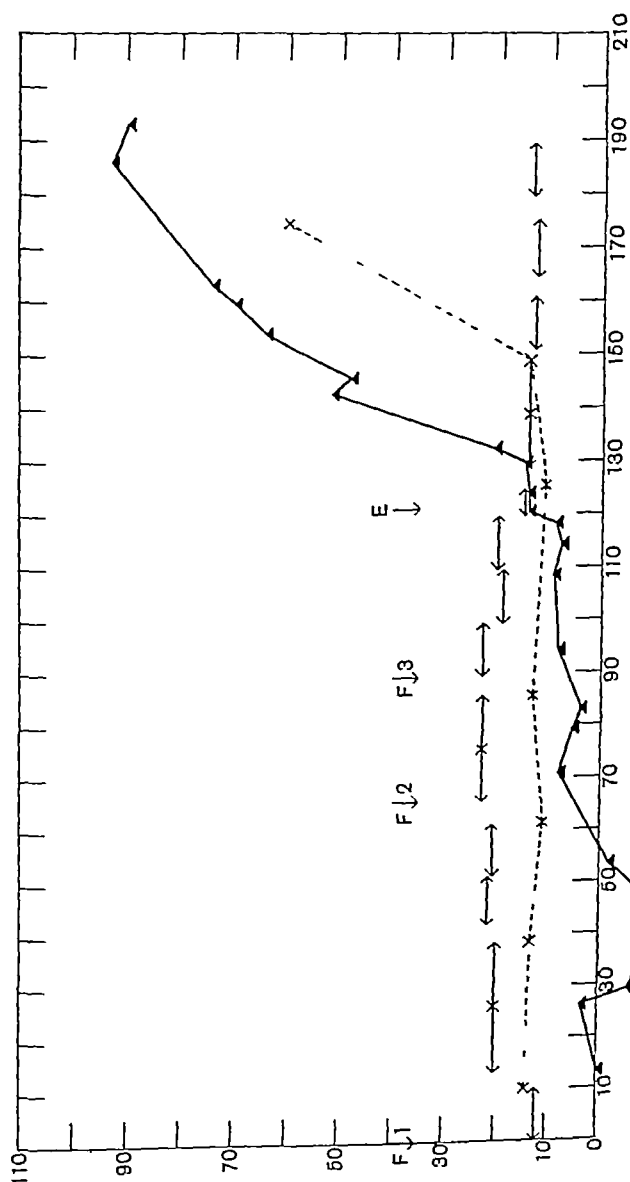
Fig 7 Perfusion begins with 3 p c acacia plus 20 p c stock horse serum in 0 05 p c NaCl containing pituitary extract 250 parts to 1,000 000 of perfusate (F1) F 2 is the same fluid without pituitary extract. F 3 is 3 p c acacia plus 5 p c stock horse serum in 0 05 p c NaCl containing pituitary extract 350 parts to 1 000 000 of perfusate Charting similar to that in Fig 3 Pressures in Table II

gives the situation on a second injection of graphite ink after forty minutes of perfusion, and Picture 3, after a third injection of graphite ink

at the end of sixty-four minutes   Picture 4, taken five minutes after 3, shows the web with the graphite ink washed out and the melanophores expanded as a result of the pituitary extract in the perfusate (*F* 1)
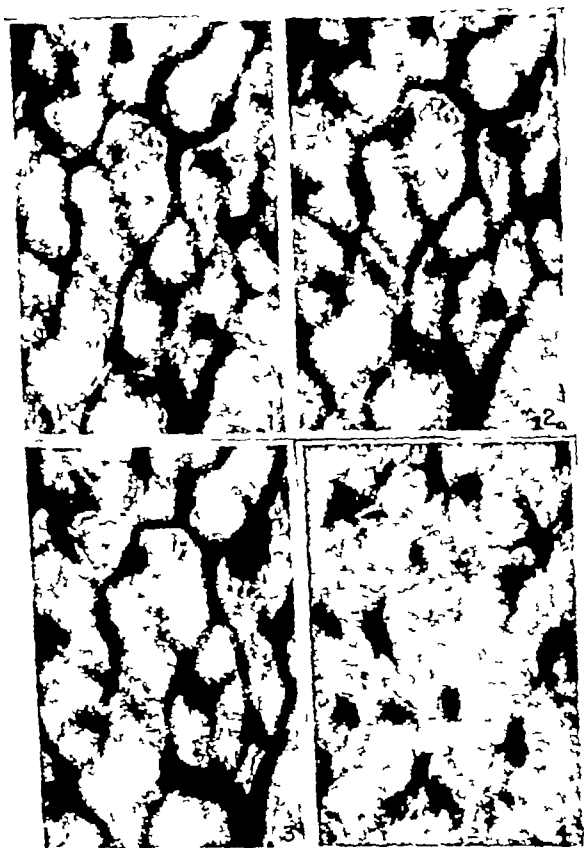
Fig 8 *A Picture* 1 Capillaries injected with graphite ink after eleven and one half
        minutes perfusion with *F* 1 (Fig 7)

Picture 2 Second graphite ink injection after forty and one half minutes' perfusion.

Picture 3 Third graphite ink injection after sixty four minutes' perfusion

Picture 4. Melanophores in same field with graphite ink washed out after sixty nine minutes'
        perfusion. Magnification × 55

The same perfusate, lacking pituitary extract (*F* 2), begins to flow on the sixty-fifth minute and is continued for twenty-five minutes   Picture 5 (Fig 8, *B*) gives the condition of the capillaries at the close of this pituitary-free perfusion   The capillaries show a slight degree of dilatation, the

rate of flow has risen (see Fig 7), and the melanophores have contracted, a fact shown better in Picture 6, taken at the ninety-second minute of
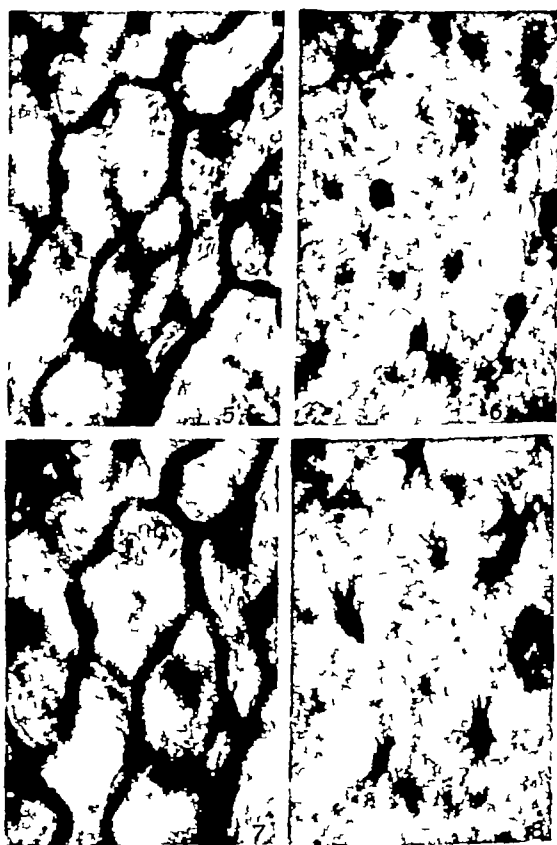
Fig 8   B   Picture 5   Fourth graphite ink injection made eighty seven and one half minutes after beginning perfusion and showing the effects of a solution (F 2) similar to F 1 but without pituitary extract

Picture 6   Made five minutes after Picture 5, when the graphite ink had been washed out.

Picture 7   Made at the one hundred and twenty eighth minute of perfusion and just at the beginning of œdema due to perfusion with F 3   Note capillary dilatation and melanophore expansion.

Picture 8   Made three minutes after Picture 7, with graphite ink washed out, in order to show the condition of the melanophores   Magnification × 55

perfusion and five minutes after 5   Perfusion with fluid 3 (F 3) begins at ninety minutes and is continued for the remainder of the experiment. Up to this time the capillaries have dilated very slightly but there

not the least suggestion of œdema Fluid 3 is 3 p c acacia plus 5 p c horse serum in 0 65 p c sodium chloride containing pituitary extract, 350 parts in 1,000,000 of perfusate This contains an inadequate serum content but is rich in pituitary extract Œdema is detectable grossly after thirty minutes of perfusion and the curve indicating swelling of the web begins to rise at about the same time and mounts rapidly Picture 7 (Fig 8, *B*) was taken on the one hundred and twenty-eighth minute of perfusion, just at the beginning of the development of œdema The significant capillary dilatation and melanophore expansion are clear The condition of the melanophores is plainer in Picture 8, taken five minutes later with graphite ink washed out Picture 9 (Fig 8, *C*) was
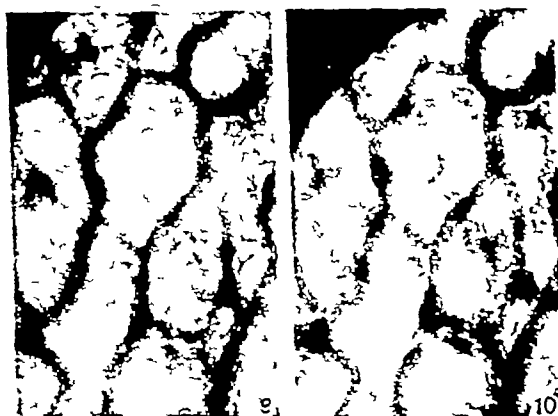
Fig 8 *C Picture* 9 Sixth graphite ink injection at the one hundred and forty ninth minute of perfusion. Œdema progressing rapidly

Picture 10 Seventh graphite ink injection at the one hundred and seventy-eighth minute of perfusion. Magnification × 55

made on the one hundred and forty-ninth minute, and Picture 10 on the one hundred and seventy-eighth minute As œdema progresses, there is a falling off in the perfusion flow, and between Pictures 9 and 10 the time required for entrance of the graphite ink into the capillaries is greatly increased These two results usually accompany œdema and are always found when œdema becomes extreme As a result of flow reduction, the melanophores are seen to have contracted Perfusion pressures for this experiment are given in Table II

When solutions contain pituitary extract in moderate amounts, it is possible to cause melanophore dilatation by perfusing rapidly, melanophore constriction by a reduction in flow, and the dilatation once more

TABLE II. MEAN PRESSURE IN THE ANTERIOR TIBIAL ARTERY
DURING THE EXPERIMENT SHOWN IN FIG 7

Time after beginning of Perfusion min	Mean Pressure cm H$_2$O
19½	33 4
32¼	34 1
51¼	34 5
61¼	34 6
78	35 1
95½	35 2
112¼	35 2
132¼	35 3
157½	36 6
174¼	35 1
188	35 1

by an increase in flow   Such a result points to the rapid destruction of the melanophore-expanding principle in pituitary extract   Indeed, given a constant rate of delivery of this substance from the gland and a constant low concentration in the blood of the frog, it seems possible that the expansion and contraction of the melanophores may depend on the amount of freedom of passage of the substance through capillary walls—conditions themselves dependent upon rate of flow, capillary pressure and capillary diameter

DISCUSSION

The experiments reported in this paper were performed upon a single group of frogs between January 14 and 24, a period not far from the mating season of brown frogs kept indoors   The results are, however, in accord with work done in November and December and undoubtedly give a true indication of the facts to be elicited from the preparation described   In order to control the pituitary extract, the results have also been verified with Parke Davis pituitrin

The experiments entirely confirm the finding of Krogh and Harrop[1], that serum possesses a peculiar power of restraining capillary dilatation and capillary leakage   When one adds a sufficient amount of serum to an acacia solution, the capillary membrane in contact with the mixture retains its normal semi-permeability and does not display the utterly free leakage which appears when acacia alone is used   The question, however, as to whether the protective effect of serum can be explained upon simple mechanical grounds or whether it depends upon unique properties inherent in serum merits discussion

The important unmeasured factor in my perfusions is the capillary pressure and further work including such measurements must be done The pressures in the anterior tibial artery, however, have been in the

range obtaining for the frog under normal circulatory conditions   With the Hess viscosimeter, the viscosity of a 3 p c acacia-Ringer solution, referred to water, is 1 75, that of a 3 p c acacia-Ringer solution plus 20 p c horse serum is 1 85—a very slight increase   If, in a perfusion, one shifts from the first of these solutions to the second, keeping the delivery pressure constant, the flow falls slightly, but one finds it impossible to believe that the insignificant increase in viscosity which the 20 p c serum confers upon the 3 p c acacia can protect against leakage by a simple process of lowering capillary pressure   In considering the protective influence of serum, one turns naturally to the constrictor effect of serum upon arterioles   The facts that one can prevent œdema with serum when the perfusion pressure is pushed very high, and that one does not destroy this protective power against œdema and capillary dilatation by the addition of acetyl-cholin to serum-containing perfusates negate the possibility that the serum operates by lowering capillary pressure through constriction of arterioles

Following upon the evaluation of horse serum as a substance peculiarly capable of maintaining capillary tone and preventing œdema, a number of experiments have been made to determine whether the effect of the serum is inherent in the serum proteins or is separable from them   While these attempts indicate that probably the effective agent is inherent in the serum proteins, the condition of the frogs available during and immediately after the mating season has been so poor as to make any final conclusion unsafe and to cause postponement of this obvious development of the work   Given a preparation such as that described, one can readily make systematic evaluations of œdema prevention and the earlier steps in localising the œdema-preventing agent, either in the serum proteins or in ultrafiltrates from serum, should present little difficulty

In regard to the failure of pituitary extracts to prevent œdema and capillary dilatation in the present experiments as compared with the efficacy of these compounds noted in former experiments from this laboratory, no final explanation can be given   If the photomicrographs of capillaries in Figs 5 and 8 are measured, it will be found that, under the conditions of perfusion used, the web capillaries were somewhat dilated, practically from the start   If one compares the viscosity of a 3 per cent acacia-Ringer-pituitrin solution, such as I have employed, with the same solution containing one-third by volume of red cells—the perfusate used by Krogh and Harrop[1]—one obtains 1 75, referred to

18—2

water, for the simple solution and 2 83 for the solution containing red
cells   If the solution containing red cells is perfused through the anterior
tibial artery, it flows at a rate about one-third that of the simple solu
tion   There is, consequently, the possibility that capillary pressures in
perfusions such as mine are higher than those in perfusions in which less
viscous solutions are utilised   For the time being, no attempt has been
made to make the measurements of capillary pressure necessary to clear
up the experimental discrepancy between the two sets of results, since
we have thought it more important to develop an objective method of
observation of capillaries and to evaluate the effects of serum upon these
vessels

SUMMARY

A new method for perfusing the foot of the frog through the anterior
tibial artery is described   This method permits the measurement of total
rates of flow, of the onset of œdema, of flow in the capillaries, and of
capillary diameter, these last two measurements depending upon the
employment of perfusates containing colloidal graphite   The procedures
described can be carried through in different animals with a degree of
uniformity and control which permits the use of the method for systematic
studies on capillary dilatation and œdema

A group of experiments is presented by means of charts and photo-
micrographs   These cover results obtained with 3 p c acacia-Ringer
solutions to which horse serum was added in 5, 10, 15 and 20 p c
concentrations   With 5 and 10 p c horse serum, capillary dilatation
and true œdema occur and the addition of pituitary extract, 200 to 400
parts in 1,000,000 of perfusate, does not abolish this result   When 15 p c
concentration of horse serum is reached, extensive capillary dilatation
and œdema occur but seldom, with a 20 p c concentration of serum,
these phenomena are practically absent

Figures are given for the colloid osmotic pressure of the solutions
used   There is no evidence that the favourable effect of serum is due to
simple increase in the osmotic pressure of the perfusates

By means of these experiments, the power of serum to maintain
capillaries in normal condition is graded in concentrations of serum
This effect is not due to a constricting action of serum on the arterioles
since it is present when the rate of perfusion flow is pushed very high and
also when acetyl-cholin is added to the perfusate

by him, to Dr Rehberg for his help with the technique of capillary photography and to Dr F Nakasawa for many determinations of colloid osmotic pressure

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THE VARIATION IN THE UNIT OF THE ŒSTRUS-PRODUCING HORMONE

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THE introduction of the vaginal smear test on ovariectomised rats or mice for extracts containing the œstrus-producing hormone has led to its widespread use at the present time for the determination of the relative potency of different preparations Doisy, Ralls, Allen and Johnston[1] have defined as a "Rat unit" the "quantity of material necessary to induce œstrus as judged by the smear method in an ovariectomised, sexually mature rat weighing 140 ± 20 grm" It is evident that the value of their unit is dependent on the assumption that the smallest dose able to produce œstrus in one rat will not seriously differ from that required in another

Certain observations made by one of us in an attempt to determine the mouse unit of different preparations, suggested that for ovariectomised mice at least this assumption was incorrect Three results, which were simultaneously obtained, appear in Table I The results with all

TABLE I

Extract No 1			Extract No 2			Extract No 3		
Mouse	Dose	Result	Mouse	Dose	Result	Mouse	Dose	Result
1	40 mgm	Œstrus	7	40 mgm	Œstrus	13	40 mgm	Œstrus
2	20 ,,	,,	8	20 ,	Nil	14	20 ,,	Nil
3	15 ,	,	9	15 ,	Œstrus	15	15 ,	,
4	10 ,,	Nil	10	10 ,,	Nil	16	10 ,	
5	7 5 ,,	Œstrus	11	7 5 ,	Œstrus	17	10 ,,	
6	5 ,	Nil	12	5 0 ,	,,	18	5 ,	Œstrus

three extracts were irregular, but consideration of those obtained with Nos 2 and 3 showed that the method applied in this way offered no hope of arriving by the use of a few animals at a trustworthy determination of the minimum effective dose It was evidently necessary to carry out an investigation of the variation between different animals

Trevan[2] has shown that tests such as the frog test for digitalis and

the mouse test for insulin must, if grave errors are to be avoided, be carried out in such a way that the variation of different animals is not neglected but is made the basis of the test   He has shown that since frogs vary in their response to digitalis by 300 p c , an effort to determine the minimal lethal dose by giving a series of diminishing doses cannot be expected to give a correct result   It is necessary first to determine for the species of frog used a standard curve relating the percentage mortality of frogs to the dosage   Having obtained this standard curve, it is then possible to assay an unknown sample by injecting *e g* 40 frogs with one dose The potency of the sample is then determined by noting the abscissa on the standard curve corresponding to the percentage mortality which occurs

In this paper we have applied the principles laid down by T r e v a n to the investigation of the rat and mouse unit, and for this purpose have examined one sample of ovarian extract on 90 rats and 70 mice

METHODS

1 *Selection of animals* One hundred young does were obtained from an average healthy stock of rats of mixed black and white strain They were 7–8 weeks old and weighed 60–100 grm They had been fed from weaning on a mixture of crushed oats 25 p c , barley meal 25 p c , middlings 35 p c , and a proprietary food called vitamealo 15 p c , with about three drops of cod liver oil per rat twice a week After being brought to the laboratory the same diet was continued, with the daily addition of about 1 cub in of bread which had been soaked in milk and squeezed out The addition of about six drops of a crude cod liver oil to the mixed diet twice a week was continued throughout the experiment   Growth proceeded at an average rate to maturity   Of the hundred animals the complete series of observations was made on 90

2 *Examination of smears* The vaginal smears were examined by the method described by L o n g and E v a n s (3), which one of us (K H C) had the privilege to learn in Prof E v a n s' laboratories in California The smear is rubbed from the spatula on to a drop of tap water or saline on a microscope slide and the constituents are identified through the low power objective at once No drying or staining is necessary The smears from 18 to 20 rats can be made in separate drops of water on one large slide, and one hundred rats can be examined by two workers in half an hour

3 *The removal of the ovaries* The operations were carried out under deep anaesthesia with ether and with aseptic precautions The rats

recovered completely within an hour, and their wounds healed by first intention in every instance   It was interesting to note that in several rats œstrus appeared within two days, but never more than two days, after ovariectomy

4   *The œstrus-producing hormone used*   The preparation of œstrin (as Parkes and Bellerby [4] suggest the hormone should be called) was very kindly placed at our disposal by Mr F H Carr, C B E , F I C, of the British Drug Houses   It was a bulk of about 10 grm of extract which was kept by Mr Carr *in vacuo* over sulphuric acid at 0° C   A day or two before each injection was made, a portion of the bulk was weighed in a tared glass tube, and olive oil was added to a mark, so that the weight of extract per c c of oil was accurately known   For injection a known volume of oil was emulsified in a mortar with 1 p c anhydrous sodium carbonate in sufficient amount to allow the desired weight of extract to be injected in a volume of 0 4 c c for every rat, and of 0 2 c c for every mouse   The dose was administered under the skin as a single injection   Local disturbances at the site of injection in the form of induration or necrosis were not observed, and there was no indication of incomplete absorption of any dose

5   *The determination of œstrus*   The change in the smear which was considered sufficient to indicate that œstrus had resulted from an injection of the extract was either (*a*) the absence of leucocytes, and their replacement by either nucleated or a mixture of nucleated and cornified cells on one day, followed on the next day by a mixture of leucocytes and cornified cells, or (*b*) the appearance of nothing but cornified cells on one day   These changes, when they occurred, were complete within 72 hours of the injection

Experimental results on rats

A small portion of the extract used was first tested by injection into five ovariectomised rats other than the rats already described   Of two of these which received 2 5 mgm. of the extract, one passed through the œstrous cycle   It seemed likely that an injection of 5 0 mgm would lead to the occurrence of œstrus in the majority of rats, and this dose was prepared for the first injection   All the rats were injected with this one dose at a time six days after the last and fifteen days after the first of the operations for ovariectomy had been performed

After an interval of 10 days, another dose was chosen and given to every rat   At successive intervals of 14 days other doses were given in the same way   The results appear in Table II

TABLE II

Date	Dose per rat	No of rats	No in which œstrus was observed
9th Dec.	5 0 mgm	90	32
19th Dec.	2 5 ,,	90	7
3rd Jan.	7 5 ,,	90	31
17th Jan.	12 5 ,,	90	55
31st Jan.	17 5 ,	90	76
14th Feb	10 0 ,,	90	40

It was a matter of considerable surprise to find that the injection of 5 0 mgm was followed by œstrus in only 32 rats The result of injecting 2 5 mgm. was consistent with this, but the injection of 7 5 mgm was followed by œstrus in one less rat than the injection of 5 mgm. The impression was given that a loss in sensitiveness had taken place The very slow increase in the number of rats giving the response as successively larger injections were made further suggested that there was a progressive loss of sensitiveness, but the result of the injection of 10 mgm. on 14th Feb showed that so far as the period following 19th Dec was concerned there had been no loss of this nature For when a curve (see Fig 1) was drawn through the points obtained by plotting the dose
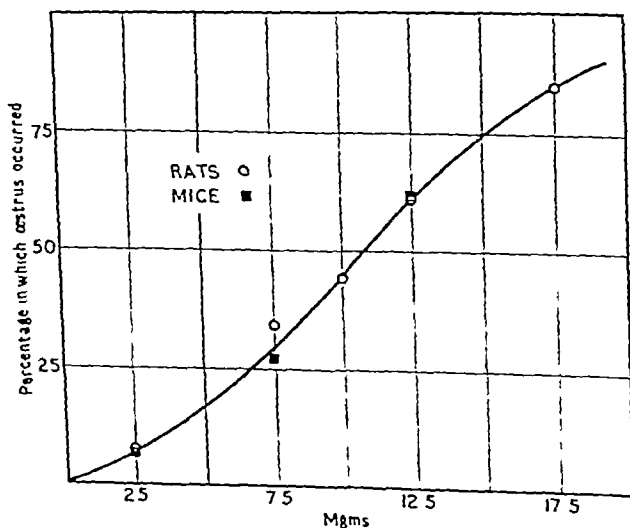
Fig 1

against the percentage of rats in which œstrus occurred, it was found that all points except that of 9th Dec lay on a well-defined curve Had there been any progressive decrease in sensitiveness the injection of

10 mgm on 14th Feb would have given a result which when plotted would have lain below the curve It has already been mentioned that the first injection followed ovariectomy by only six days in some of the rats, whereas the remaining injections were separated by 14 day intervals Analysis of the figures showed that the disagreement between the result of the first injection and that of the later ones was due to this difference, and that for the purpose of establishing the variation in rats, the result of the first injection must be set aside as being complicated by an additional factor

The coincidence of the result of the injection of 14th Feb with the predetermined curve, showed in addition that there had been no loss of activity of the extract used during its stay *in vacuo* at $0°$ C

The results showed that not only was it untrue that the minimal effective dose of œstrin was approximately the same for all rats, but that the divergence between different rats was unusually wide Seven times the amount of œstrin sufficient to produce œstrus in 7 p c of animals, was still insufficient to produce œstrus in more than 84 p c of animals

EXPERIMENTAL RESULTS ON MICE

Several observers have preferred to use the mouse, and it seemed of importance to make parallel observations on this animal to determine with accuracy (*a*) whether mice showed the same variation as rats, (*b*) the relation of the mouse unit to the rat unit The experiments were performed on 70 mice, all obtained from one dealer at one time, in exactly the same way as those on the rats The operations for ovariectomy were completed on 20th Dec and the first injection was not made until 14 days afterwards As before, 14 days intervened between successive injections The œstrin was prepared as for the rats, the material injected being a portion of the sample used for injection into the rats on the same day

The results appear in Table III

TABLE III

Date	Dose per mouse	No of mice	No in which œstrus occurred
3rd Jan	2 5 mgm	70	5
17th Jan	7 5 ,	70	19
31st Jan.	12 5 ,	70	43

It will be seen from Fig 1, that when these observations are expressed as a percentage of mice and plotted against the dose, the points lie on the same curve as that given by the rats

RELATION OF VARIATION TO BODY WEIGHT AND OTHER FACTORS

The doses of œstrin injected were not calculated according to the body weight of each animal either for rats or mice. It seemed possible, at first sight, that the variation observed between rats might to some extent be due to this, and that on analysis it might be found that it was lighter animals only which responded to the lower doses. Each rat had been weighed once a fortnight throughout the experiment, and examination showed that there was no correlation between body weight and the dose necessary to produce œstrus. Thus the injection of 12 5 mgm of the extract on 17th Jan produced œstrus in 55, and failed to do so in 35 rats. The average weight of the 55 rats was 150 grm, the average weight of the 35 was slightly, but not significantly less. The weights in the two groups were distributed fairly evenly about the same weight, 150 grm

The group of rats in which œstrus occurred had on the whole grown rather more in the previous six weeks, but the difference was again not significant

Vaginal smears of all the rats had been taken daily for three weeks before ovariectomy, and during this period some rats were seen to pass through one or more cycles while in others no cycle was observed. It was not found after ovariectomy that the former group was the more, or that the latter was the less sensitive to œstrin

REGENERATION

A factor which might have complicated the results was regeneration of ovarian tissue after ovariectomy. Smears were taken daily in rats from the time of operation for 34 days. For the next six weeks they were examined only on the day before injection and the next five days. From this point they were examined daily for 25 days. Regeneration was observed in two rats only, and these are not included in the 90 animals on which the results were obtained. During the injection of the mice, smears were again only examined on six days, 14 days after the last injection, a daily examination was made again for 25 days, there was evidence of regeneration in five mice only, which is a much smaller number than had been expected from the results of Davenport[5]

INTRAPERITONEAL INJECTION

Evans and Burr[6] have published a short paper giving results on a few animals from which they conclude that the dose necessary to

produce œstrus is larger if it be injected into the peritoneal cavity than if injected under the skin As they were apparently unaware of the great variation in rats, it was possible that their result was merely a demonstration of this variation, and not of the difference between the two modes of administration The hypodermic injection of 10 mgm. of the extract into the rats on 14th Feb produced œstrus in 44 p c On 28th Feb this amount was administered by intraperitoneal injection, and œstrus was produced in 48 p c The difference between the two results is not significant, and the results show that it is immaterial whether the injection be made under the skin or into the peritoneal cavity

INJECTION IN THREE DOSES

Evans and Burr (*loc cit*) give illustrations confirming the previous suggestion of Doisy, Ralls, Allen, and Johnston (*loc cit*) that a smaller amount of extract is sufficient to produce œstrus if it be injected in three separate doses at intervals of four hours, than if it is injected in one single dose

Fourteen days after the intraperitoneal injection just described, 50 rats received by hypodermic injection a total of 10 mgm given in three doses at four-hour intervals Œstrus followed in 19, that is in 38 p c This figure is lower than that obtained by a single injection of 10 mgm into 90 rats, which was 44 p c , but here again the difference is not significant It is evident that the single is at least as efficient as the triple injection

THE VARIATION IN THE RESPONSE OF SINGLE RATS

In view of the variation in the sensitiveness of different rats, it was most important to discover whether each animal behaved in a reasonably uniform way The evidence on this point is on the whole depressing In the first place the behaviour of the rats may be considered in giving the results which appear in Table II If the result of the first injection be excluded for the reasons already stated, then in the course of the other injections 30 out of the 90 animals behaved irregularly, in the sense that doses higher than the smallest which produced œstrus did not invariably produce œstrus From the 60 rats, which were regular in this sense, should be deducted 14 in which œstrus never occurred at all, so that out of 76 animals which may be used as evidence, three-fifths were regular and two-fifths were irregular

Further information is given by the three injections each of 10 mgm The second of these was an intraperitoneal injection, and for the third

three injections of 3 3 mgm were given at intervals of four hours Since the results were fairly uniform (œstrus occurred in 44, 48 and 38 p c respectively) it may be assumed that the method of injection made no difference, and that the same results would have followed a single hypodermic injection in each instance If this be granted, then of the 50 rats which were used in all three observations, only 19 gave the same response each time For the first two observations 90 rats were used, and in these 51 animals behaved in the same way both times It is clear that the sensitiveness of any one animal undergoes very considerable variations

THE METHOD OF ASSAYING AN EXTRACT

The rat unit may be redefined as that amount of extract which produces œstrus in 50 p c of ovariectomised rats In order to examine an unknown sample, 20 rats are chosen from the stock, 10 of which do not usually respond to an injection of one unit, and 10 of which usually do respond to this amount To each of the 20 rats the same dose is given, and the number in which œstrus occurs is observed Suppose this be x, so that the percentage is $5x$ The abscissa corresponding to the ordinate
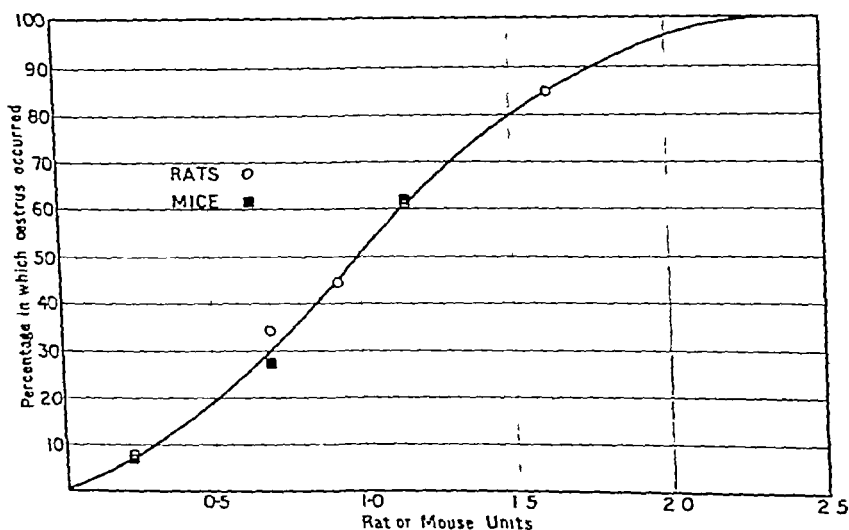
Fig 2 Standard curve showing relation of units injected to effect produced.

$5x$ is then noted on the curve shown in Fig 2 This abscissa gives the number of units present in the dose injected.

The rats used for this assay are injected every fourteenth day, and at

no other time There is at present no trustworthy evidence to show the effect on sensitiveness of discontinuing the injections for any period

DISCUSSION

It has been found that the variation in the rat unit as defined by Doisy, Ralls, Allen and Johnston (*loc cit*) is more than 700 p c and appears from the curve shown in Fig 2 to be about 1000 p c That this great variation is not due to accidental selection of unusual animals appears from the fact that precisely the same variation has been found in the mouse unit From the investigation has emerged the very striking observation that the average rat unit is the same as the average mouse unit This fact is significant for the understanding of the action of œstrin. That the same amount of œstrin which just suffices to induce a response in a mouse should also be enough to bring about the same change in an animal 7 5 times as big, differentiates its action sharply from that of insulin, the dose of which is closely proportional to the body weight It is clear that the factor which determines the size of the minimum effective dose is not the necessity for a certain concentration of the hormone in the body tissues or fluids

The variation in different animals greatly complicates the assay Provided however that different workers determine the average rat or mouse unit in the manner described, there should be a certain degree of resemblance between the results of different workers, inasmuch as rats in different countries are not likely to be more dissimilar than rats and mice in Great Britain It should be recognised, however, that even if the average unit be determined, unless this is done on approximately 100 animals, there may still be serious discrepancies between different workers, and these will remain until a standard sample of œstrin is available for distribution by a central international authority When this is prepared it may be prophesied that by a general consensus of opinion the unit will be redefined as the amount of activity present in a certain weight of the standard preparation Having determined in 20 animals the number which respond to a given dose of the standard, the strength of an unknown sample will then be determined in terms of the standard by injecting the 20 animals with a dose of that sample

The variation which may occur in determining the average unit at the present time can be calculated from the formula for the standard deviation $\sqrt{\frac{p \cdot q}{N}}$ where p is the percentage of animals in which œstrus has occurred, q is the percentage in which it has not occurred, and N is

the number of animals   If 25 animals are used, and œstrus occurs in 50 p c , the standard deviation will be 10 p c   This means that if the experiment be repeated several times, on different rats, then twice out of three times the percentage of animals in which œstrus occurs will be not less than 40 or more than 60   But once in twenty-two times the percentage will differ by more than twice the standard deviation, and be less than 30 or greater than 70   Thus if only 25 animals are used, once in twenty-two experiments a dose which is actually one average unit will appear as less than 0 7 units or as more than 1 3 units, and the error in the assay will be more than 30 p c

SUMMARY

1   An investigation of 90 rats and 70 mice has shown that the variation in the rat unit or the mouse unit may be as great as 1000 p c

2   The unit is redefined as the dose required to produce œstrus in 50 p c of ovariectomised animals

3   The relation of the average rat unit to the average mouse unit is one

4   The average unit is the same whether the injection be made as a single subcutaneous injection, as a single intraperitoneal injection or as a triple subcutaneous injection of which the separate injections are made at intervals of four hours

5   Single animals, injected once a fortnight with the same dose, vary considerably in their response

6   The method used in this laboratory for determining the unit is described

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STUDIES ON NERVE METABOLISM   I   The influence of oxygen lack on heat production and action current

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IN previous papers (1, 2, 3) it has been shown that activity of nerve is associated with a definite heat production, and the absolute value for a single impulse obtained This heat appears in two phases, an initial very short and intense one associated with the actual conduction and immediate restitutive processes, and a delayed feeble but very prolonged one associated with "recovery" processes In the case of muscle, which shows two similar phases, the delayed heat is almost all oxidative, since it disappears in oxygen lack, whereas the initial heat is non-oxidative, being independent of oxygen supply It was expected that a similar mechanism would be found in nerve, possibly based, as in muscle, on a glycogen-lactic acid system The present research represents an attempt to expose the nerve mechanism, and the results obtained do not support this hypothesis, but do not positively eliminate it The problem is being followed further by chemical methods

Especially interesting is the evidence that for minutes after activity a nerve has not returned to rest—not only does extra heat production last long after conduction is over, but potential or permeability changes may also considerably outlast the transmitted impulse (see Levin[4] and Verzar[5]) The dependence of these electrical changes on the presence of oxygen has been much studied but the results can hardly yet be given a sound interpretation

METHOD

These experiments were carried out during the same period as those already reported, and the two galvanometer arrangements described (1) and (2) were both used The general arrangement of the electric circuits was also as described

In a large number of experiments the action potential as well as the heat was recorded The former was led off by electrodes (3) and (4) from

the uninjured side and crushed end respectively to a moving coil galvanometer The electrodes were ordinary silver ones and easily polarisable, but this did not matter, for in series with the galvanometer in the "circuit' was a 10 mf condenser Any change in E M F would tend to charge (or discharge) the condenser and in so doing produce a pulse of current through the galvanometer which would respond with a ballistic throw The large capacity of the condenser used made its time of charge and discharge relatively long so that very rapid changes of E M F were averaged, and it also insured a relatively large current pulse when being charged by a low E M F By tetanisation of the nerve, a regular series of action potentials was produced and the condenser charged to the average potential A quantity of current proportional to the change of potential surged through the galvanometer and gave a ballistic throw that directly measured the average potential developed When tetanisation was stopped the potential would return to zero (or rather to the original injury potential) and the condenser discharge through the galvanometer, giving a deflection in the opposite direction Except for minute leaks in the condenser the total current flow was nil, and the galvanometer zero steady, as the slow changes of injury potential would not be perceptible The galvanometer deflections during charge and discharge of the condenser must be equal and opposite if the system has returned to its initial state As a matter of fact under ordinary conditions the throw when stimulation was stopped was one-third to one-half again as great as that when it was begun This may have been partly due to a gradual increase of action potential from one impulse to the next so that the condenser continued to charge for a time after the main surge of current, whereas the discharge of all the accumulated potential did occur in one surge Another factor may have contributed to this effect, however, for after the negative variation ends the nerve does not return to zero potential but rather "overshoots' to a positive variation The effect is absent when a nerve has begun to fail in nitrogen charge and discharge becoming equal

The galvanometer used, a high resistance high sensitivity (1 mm = 2×10^{-10} amp) Cambridge moving coil instrument, had a very slow period (8 sec one way) and was therefore able easily to sum the short currents produced Deflections of about 100 mm were obtained at 3 metres distance

Only spring and autumn Dutch *esculenta* frogs were used, the sciatic nerves prepared as before Their arrangement on the thermopile however, was different, in that the groove containing the "hot" junctions

was not filled with nerves flush to the level but only lined with a layer of them one deep, and each nerve in contact laterally with its neighbours This gave, in effect, a plate of nerve tissue lying on the thermopile and made possible more certain control of the gas diffusion factor in oxygen deprivation experiments   In one experiment the nerves were laid on the thermopile in the usual manner and then completely covered, except at the end for stimulating, with a layer of white vaseline over 1 mm thick, to delay gas diffusion—this procedure had no effect on the results   In many of the experiments, to ensure true monophasic action currents, the entire bundle of nerves was tightly ligatured between the thermopile and electrode 4

The special Harvard coil giving 140 make and 140 break shocks a second was used throughout, and stimuli were usually of 10 secs duration Stimulation was always repeated at regular intervals of 1, 2, 5 or 10 min to avoid irregular "fatigue" effects   The arrangement of the nerves and electrodes in the chamber was such that the stimulated end was in the same gas as the remainder of the nerve   It was necessary therefore to be certain that any fall in observed heat production was not due to the stimulus becoming submaximal as excitability diminished   In several experiments the maximal stimulus at different stages of asphyxia was roughly determined and then an appropriate maximal stimulus used throughout

The temperature in all experiments was about 15° C

Preparation of oxygen low medium   As ordinarily used, the nerve thermopile is covered with a large test tube, moist with Ringer and closed with a rubber stopper through which glass tubes pass   Some of these carry the necessary wires, others permit gases to be passed in and out of the enclosed space   The whole is immersed in stirred water in a vacuum flask   The space about the thermopile, within the test tube, contains air or oxygen and measures about 200 cc   To obtain an oxygen free medium, the ideal procedure would be to fill this with well boiled saline which could be displaced with pure nitrogen, but this is impossible for several reasons   In each experiment it is desirable to make observations both in the presence and absence of oxygen, and to get comparable results the thermal characteristics of the medium must remain the same, so that both must be performed in a gaseous medium   (A liquid would conduct a great deal of the heat produced away from the junctions, so much decreasing the sensitivity, and it would also surely lead to electrical leaks)   To displace the air first with boiled saline and this with nitrogen would produce great temperature changes and prohibit observations for

several hours, by which time the nerve would no longer respond to stimulation The method actually adopted therefore involved displacement of the air or oxygen by gases

Two gas conduit tubes were carried through the stopper into the thermopile chamber, one ending just within the stopper and the other reaching almost to the floor of the test tube These were connected externally through three way stop-cocks so that each tube could communicate with the outside air (when serving as outlet) or with a source of gas under pressure (when serving as inlet) The nerves were set up in air and observations taken, then hydrogen was admitted at the top, slowly at first to minimise mechanical mixing and later rapidly to sweep out remaining traces of the original gas, the tube reaching to the bottom serving as exit

After 10 min of continued passage of hydrogen, the cocks were reversed and nitrogen admitted at the bottom while the hydrogen was blown out at the top the stream being passed fairly slowly for 5 to 10 min. The hydrogen would displace air most effectively because their different densities tend to prevent mixing, but it was necessary to have nitrogen as the final medium because the thermal conductivity of hydrogen is much greater than that of air and deflections obtained in it would not be comparable to those obtained in air This procedure never gave an oxygen free medium, but in several experiments analysis showed less than 0 2 p c oxygen present in the gas It was found that five or ten times this amount of oxygen made no difference in the results, so it may be safely assumed that in the complete absence of it the results would have been the same Following the passage of these gases, the heat equilibration was soon re-established so that reliable readings could be obtained in 10 to 20 minutes

The gases used were themselves purified of oxygen. The hydrogen was taken directly from a commercial cylinder and bubbled through alkaline pyrogallol or hydrosulphite and washed in alkali. This was sufficient because the commercial gas has a very low oxygen content and the whole was subsequently replaced by nitrogen Commercial nitrogen contains 2 p c or more of oxygen which could not be removed by bubbling through absorbing solutions An airtight system of glass tubes and large reservoirs therefore was constructed so that nitrogen from a cylinder might bubble through several pyrogallol chambers and also remain for several days standing over a surface of the same solution. The nitrogen finally obtained in this way contained 0 1 p c oxygen or less, as determined by Haldane's method, and gave clear cut results when used

To eliminate, however, the possibility that failure of the nerve in this gas might be due to something formed from the pyrogallol (e g carbon monoxide) rather than to oxygen lack, the whole system was later charged with alkaline hydrosulphite solution in place of the pyrogallol The nitrogen obtained then showed no oxygen at all on analysis and the nerves be haved just as before

In one experiment, to eliminate all possibility of direct oxidative pro cesses due to the traces of oxygen always present some hydrogen cyanide gas was passed into the chamber instead of the other gases This was generated by dropping sulphuric acid on solid sodium cyanide in a test tube and led into the chamber as usual The outlet tube dipped into strong alkali to absorb any cyanide leaving the chamber The results were again like those in nitrogen

RESULTS

(a) *Initial heat* For a 10 sec stimulation of nerves, the maximum deflection of the galvanometer gives the total initial heat directly, so a curve connecting successive maxima obtained by regular repeated stimulation indicates directly any changes in the initial heat Fig 1 contains
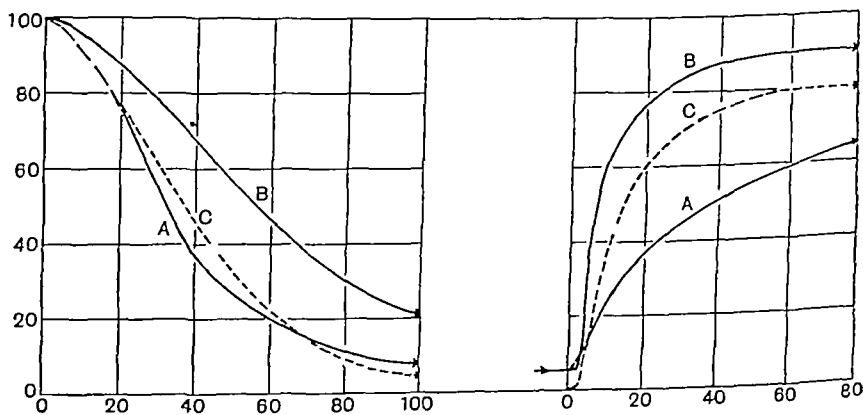
Fig 1   Failure of (A) initial heat and (B) action potential [(C) = action potential squared] in nitrogen and return in oxygen. Vertically, maximum deflection of galvanometer in p c of the initial value horizontally, time in nitrogen or in oxygen following nitrogen. The dashed portion of (A) is interpolated

such curves showing the failure of initial heat when a nerve is deprived of oxygen and its return when oxygen is re-admitted The curves pass through mean points obtained by averaging observations from over ten individual experiments, all of which gave consistent results The first

portion of each curve (dashed) is interpolated, since no observations can be made at once after changing the gases

Twenty minutes after the displacement of oxygen is begun (five minutes after it is finished) the heat has fallen to three-fourths of its initial value It continues to fall rapidly at first and then more slowly, to 7 p c of its initial value in 100 min and to zero in about three hours If oxygen is then admitted, heat production on stimulation is resumed within a few minutes and increases for an hour or two The final equilibrium value attained is occasionally just as high as the original one, but usually is less, about 20 p c less on the average

In two experiments the nerves were asphyxiated and allowed to recover twice In one, during the first asphyxiation they were tetanised for 30 sec every min , and during the second asphyxiation for 10 sec every 10 min In the other experiment this order was reversed It was hoped to show in this manner that, when more active, nerve heat failed in nitrogen in a shorter time than when almost resting There was no clear cut difference in either case, though probably the frequent stimulation did hasten complete failure of response

The rate of recovery of heat production after oxygen was re-admitted into the chamber seemed definitely to vary with the time the nerves had been in nitrogen If the nerves were kept in nitrogen two or three hours after heat production had completely failed, recovery in oxygen was slower than if oxygen was admitted shortly after failure was complete Due possibly to the time factor, recovery was much more rapid when oxygen was admitted before complete failure than when admitted after it No measurable recovery followed the admission of small amounts of air until the gas contained 2 to 3 p c of oxygen. (One experiment)

(*b*) *Recovery heat* This is determined from the total area of the galvanometer deflection-time curve In a normal production of heat the ratio of the total area (in mm –sec) to the maximum deflection is constant at about 190 As the time in nitrogen increases the maximum deflection steadily falls and the area also falls, but the ratio remains unchanged The general shape of the curves in air or nitrogen also remains the same (Fig 2), though careful recording shows a slight delay in the nitrogen curves reaching the maximum deflection (Fig 3) The small difference in maxima remains after oxygen is re-admitted in cases where the nerve heat does not return to its full original value, and is due to failure of the deeper fibres next to the junctions to produce heat The heat produced by other fibres must therefore be conducted through them and is so delayed This does not modify the general fact that the total

heat remains in quantity and time distribution in essentially constant relation to the initial heat The recovery heat, then, fails in lack of oxygen,
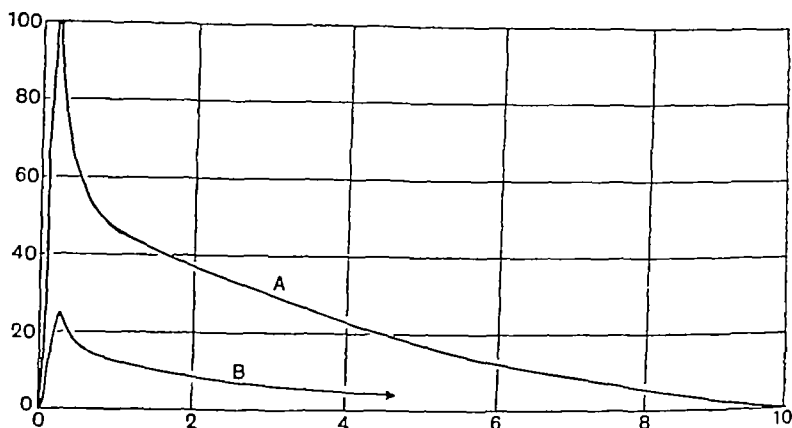
Fig 2. Vertically, deflection of galvanometer in arbitrary units horizontally, time in minutes. Curves for 10 sec stimulation (*A*) in oxygen, (*B*) after 1 hour in nitrogen The general shape of the curves, corresponding to heat production, is the same in both.

but only in so far as the initial heat that it follows is failing This statement may not be exact in its comprehensiveness, for the error in taking
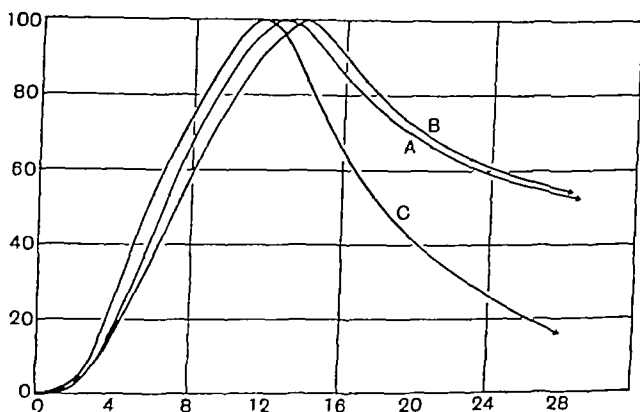
Fig 3 Vertically, deflection of galvanometer in p c. of maximum horizontally, time in seconds Curves for 10 sec stimulation (*A*) in oxygen, (*B*) after 1 hour in nitrogen or (*C*) for 10 sec. warming with alternating current through the length of the nerves after they had become entirely inactive in nitrogen

total heat curves in nitrogen is considerable (due to drifts, the continually changing state of the nerve, and the relatively few observations that can

be made and averaged in each experiment) It is impossible to exclude discrepancies of, say, 25 p c in the ratio of initial to total heat in nitrogen, but if such a change in the ratio exists it is certainly an increase, i e there may be somewhat more delayed heat in proportion to initial in nitrogen than in air This is suggested by the results of prolonged stimulation

When the nerves are tetanised in air or nitrogen for 5 to 10 minutes, or warmed after becoming inactive in nitrogen by passing a weak alternating current through their length for a similar time, heat curves like those shown in Fig 4 are obtained The control warming curve has reached
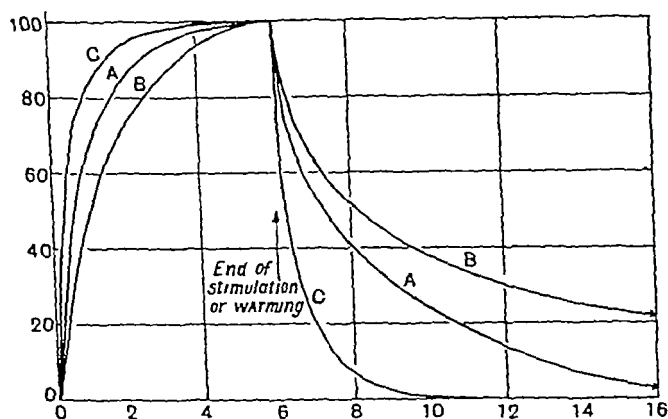
Fig 4 Vertically, deflection of galvanometer in p c of maximum horizontally, time in minutes Curves for continued stimulation for 6 min (*A*) in oxygen, (*B*) after 1 hour in nitrogen or (*C*) for equivalent warming of the totally asphyxiated nerve

90 p c of its maximum deflection in one and a half minutes after warming starts and correspondingly lost 90 p c of its deflection one and a half minutes after warming ends For stimulation in oxygen the deflection is only 78 p c complete in one and a half minutes after beginning stimulation, which means that relatively more heat is being produced after this time than in the control The latter represents heat production at a constant rate, so that the nerve must be giving heat at an increasing rate, in other words, the delayed portions of the nerve heat for each impulse continue to sum (until at the end of delayed heat production from the first impulses, in about 10 minutes, the rate of heat production reaches a maximum) Also because of the delayed heat, the fall after stimulation ends is less rapid than the rise, only 55 p c of the deflection being lost in one and a half minutes In the case of nitrogen, the

same effect is seen more exaggeratedly, so that if the above interpretation is correct the delayed heat is relatively more important here, that is, the initial heat has been more cut down in nitrogen than has the delayed heat, or the delayed heat has been prolonged in time   It may be noted that the delay in rise and fall of these curves could not be explained by inactivity of the deeper fibres, for the delay so introduced is only about one second   Also any falling off of heat as stimulation continued, due to fatigue, would tend to hasten the maximum deflection rather than delay it, so the difference between the curves obtained in air and nitrogen could not be due to greater fatigability in the latter   The possibility of some heat being conducted down from the stimulating electrode region is, however, very difficult to exclude in such prolonged stimulations, and if it occurred it would have a relatively greater effect in nitrogen because the live heat is less, and the effect would be to delay the maximum   It is a just possible alternate explanation of these curves

The two experiments with vaseline and cyanide have been mentioned In both of these the total heat curves retained their normal shape and area-maximum ratio   The fall of maximum deflection with time was also in general similar to that in nitrogen alone, though it was striking that under vaseline the nerves lasted considerably longer than without it

(c) *Resting heat production*   All the observations on nerve heat production so far given apply to the excess heat production of activity over that of rest   The latter cannot well be measured by these methods, for it would reveal itself only as a constant potential from the thermopile   If the system settled down to true zero, so that with a dead nerve, say, the potential always was zero, it would be simple to obtain the resting heat production from the potential when the nerve was living   The random errors are too great and equilibrium too imperfect to justify this ordinarily   When, however, a nerve has come to equilibrium in air, is then allowed to reach a steady state in nitrogen after excitability is entirely lost, and finally returns to the original equilibrium in air, it may be assumed that the difference between the two equilibria represents a change in resting heat production   If, further, it is assumed that in nitrogen all resting metabolism is stopped (an assumption approximately valid only after some hours in nitrogen, for at first metabolism surely continues, but at a diminishing rate), a rough estimate of the resting heat production in oxygen may be made   This comes out at somewhat under one-third of the excess heat due to continued activity or at a rate of $2\ 0 \times 10^{-5}$ cal per sec   The value cannot be regarded as exact, but is of interest for comparison with the resting gas exchange of nerve   This

has been measured recently under conditions nearly identical with those of these heat experiments[6], and the resting O_2 consumption is 16 c mm per gram per hour, corresponding to $2\,3 \times 10^{-5}$ cal per sec

(*d*) *Action potential* The average action potential falls with time in nitrogen, though less rapidly than does heat, and returns in oxygen much more rapidly (Fig 1) Since heat directly measures energy, and the action potential does not, a direct comparison of them is of limited value The true energy equivalent of the action currents cannot be obtained (except by integration from the exact form of the action potential curve obtained under the given conditions), but since energy varies as the square of potential, this gives a better series of values for comparison with the heat The dashed lines in Fig 1 represent the square of the action potential which more nearly, but still far from, parallel the heat curves The average action potential may be reduced by short circuiting through inactive fibres or increased by prolongation of the action potential in each fibre, so that the galvanometer records a varying fraction of the true potential changes as asphyxia progresses The disagreement with the heat values is, therefore, not very significant

In the first experiments it was found regularly that the action potential increased markedly during the first few minutes in nitrogen This was finally traced to the fact that prior to admitting the nitrogen the nerves were being stimulated every 5 or 10 min only, whereas after admitting it tetanisation was repeated every minute to obtain a good series of readings during the initial part of the fall The effect is entirely independent of the presence of oxygen and was eliminated by stimulating at the same time intervals before and after displacing the oxygen Fig 5 shows the effect of frequent or infrequent tetanisation in air—the total action potential regularly shows a "treppe" with frequently repeated stimulation This may be due to increased height of the maximum potential developed or to a longer duration of potential following each impulse or, of course, to both The method used does not permit discrimination between them, but other workers have found evidence of both effects Davis and Brunswick[7], using a string galvanometer ballistically, have observed a rise in total action current from one impulse to the next when tetanisation is begun, and Prof Gasser has informed me that the oscillograph shows a definite rise in the maximum potential through the first few impulses of a tetanisation On the other hand, Tigerstedt[8], Borruttau[9], and other workers have found that the descending limb of a single action current becomes definitely prolonged and the maximum lower under conditions which might be regarded as

leading to fatigue or injury—continued stimulation, asphyxia, cold, etc
It is difficult, therefore, to know how to interpret this effect, as a "fatigue"
setting in very early or as a "facilitation " The behaviour of heat pro
duction with different intervals of tetanisation gives a clue to this,

Fig 5 Vertically, maximum deflection of galvanometer in arbitrary units horizontally,
time in minutes (A) initial heat and (B) total action potential produced by 20 sec
stimulations at 2, 5 or 10 min. intervals in oxygen The heat for each stimulus is less
and the total action potential more for short intervals between stimulation periods
than for long ones. The steady fall of heat with time, shown by the general slope of the
line is not usual

though the results have not been entirely uniform   Fig 5 shows the
results of an experiment which represents the course of heat production
in most cases, and shows a clear cut falling off with frequent stimulation,
but in a few experiments this effect has been absent for no discovered
reason   Field and Brucke(10) have found that the absolutely re-
fractory period of nerve may be increased ten times during ten minutes'
continued tetanisation, and observations on the excess oxygen con-
sumption of a nerve stimulated at different intervals point in the same
direction (6)   The conclusion seems justified, therefore, that for minutes
after a tetanisation lasting a number of seconds a nerve is less able to
liberate energy than before, in other words, it shows definite "fatigue"
after short activity This is also in accord with the existence of the
prolonged recovery heat lasting for ten minutes

DISCUSSION

The results given above indicate a greater dependence on oxygen than other workers have obtained Fillie[11], for example, found it impossible to asphyxiate nerves in saline containing between 0 1 and 0 3 mgm of oxygen per litre (corresponding to 0 2-0 7 p c O_2 in a gas), whereas in these experiments heat production and action potential failed in gas containing over 1 p c of oxygen It may be emphasised that no great difference in the effects of 0 2 to 1 0 p c oxygen was observed, though asphyxiation may have proceeded more rapidly with the lower concentrations The availability of oxygen to the individual nerve fibres depends, of course, on its being dissolved in the tissue fluids about them, and in isolated nerve the question of oxygen diffusion must be considered

The oxygen used by resting and active nerve respectively is 0 00027 and 0 0013 c c per grm per min (6) The amount of oxygen that could be dissolved in 1 grm of nerve exposed to air[1] is about 0 0112 c c The rate of saturation of the nerves as actually used, assuming no oxygen present at the start, may be calculated from Fourier's diffusion theory Mr F J W Roughton has kindly done this for me and the results are that they would be 17 p c saturated in 5 sec , 60 p c in 30 sec , and 80 p c in 60 sec If it be assumed that a balance between oxygen used and oxygen diffusing in must be established every five seconds (the nerve becoming entirely depleted in the process), the amount used by 1 grm of resting nerve is 0 000028 c c and this must equal 17 p c of the final content of oxygen at saturation The latter must, therefore, be 0 00017 c c and requires an outside oxygen pressure of $\frac{1}{70}$ that of air or 0 3 vol p c of O_2 in the surrounding medium This value (not allowing for activity) is much too low, for when the nerve has become 17 p c saturated with oxygen it will not be evenly distributed throughout the tissue and the deeper fibres will still not be obtaining anything like an adequate supply of the gas Also, the nerve does not alternately use up all the dissolved oxygen and then wait for more to diffuse in, as assumed, but an equilibrium between use and diffusion is established at some constant oxygen content This would considerably lower the rate of diffusion in, e g if the nerve remained half saturated the increment of oxygen during the first five seconds would be 8 p c instead of 17 p c of the final saturation value

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[1] Solubility of oxygen from air (at 15° C) in 1 c c $H_2O = 0 007$ c c , in oils about 0 038 Composition of nerve = ⅓ water ⅓ lipoid, ⅓ other solids Sp gr = 1 06 Assuming O_2 half as soluble in the non lipoid solids as in water, the amount in each of these would be, per grm nerve 0 0046 0 0004 and 0 0008 respectively, sum = 0 011

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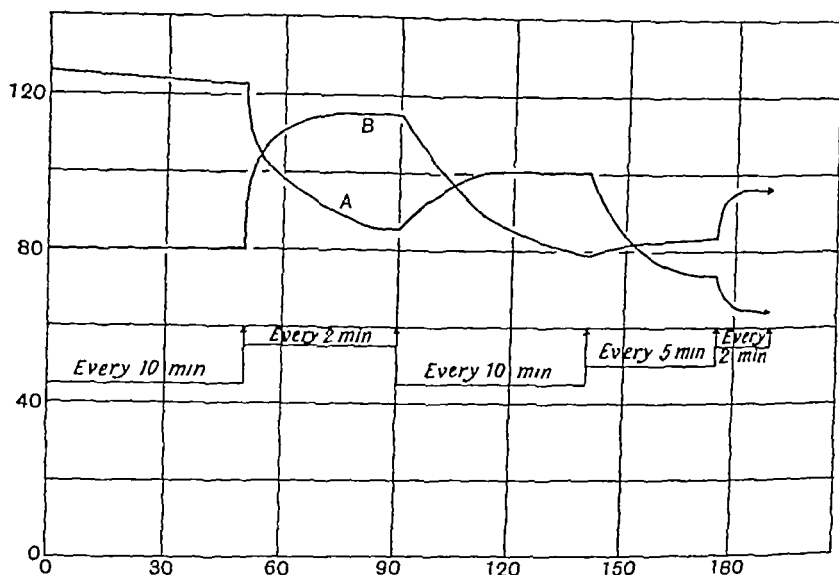duction with different intervals of tetanisation gives a clue to this,

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which it ended in its muscle The presence of a minimal muscle twitch was taken to indicate the presence of conductivity It is obvious that some fibres about the surface of a nerve will continue to receive oxygen long after the majority have been asphyxiated, and these would give a minimal contraction It is easy to estimate that in the nerves used here the thickness of the plate represents about 70 layers of individual fibres The bottom layer of fibres, therefore, receives oxygen at a concentration corresponding to 0 05 vol p c oxygen when the whole nerve is exposed to the critical 4 p c of oxygen, and at this concentration is able to function, so that fibres on the nerve surface should remain active in media containing this amount of oxygen, and probably even with considerably less The total heat and action current from such a few fibres would, of course, be immeasurable

The question next arises why the failure of nerve heat and action current in nitrogen? The total heat due to activity depends on three factors, the number of fibres acting, the frequency or number of impulses conducted by each, and the heat produced by each impulse A decrease may be due to any or all of these[1]

The possibility exists that due to lack of oxygen the immediate recovery processes following conduction (during the refractory periods) are delayed so that the nerve can respond to fewer stimuli per second Fröhlich(14) some time ago claimed that the absolutely refractory period of nerve was much prolonged in nitrogen, but Cooper(15) was unable to confirm this Even she found, however, that in the later stages of asphyxia the least interval between two stimuli for muscular summation increased several times This effect she believed due to conduction with a decrement in asphyxia so that the second impulse becomes extinguished if too early and therefore feeble, and she concluded that oxygen is not directly involved in the conduction of an impulse or immediately following Later work of Kato(16) and others throws doubt on this interpretation and the ultimate conclusion is left open It is highly probable, however, that as asphyxia proceeds the fibres cannot follow as frequent stimulation as they can in O_2

Another possibility mentioned, that for each impulse actually transmitted the heat production is less would, of course, follow from the above when tetanising stimulation is carried out, in that each impulse falls on the nerve less recovered from the previous impulse than normally and

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[1] The further analysis of these several factors in the case of heat production is very difficult though not impossible but for action potential it is relatively simply done and in a quantitative manner This will be more fully gone into at another time

A more certain estimation of the diffusion factor can be made from Warburg's(12) work   He developed an equation for the equilibrium condition when a slice of tissue having one dimension definitely smaller than the others is exposed to various oxygen concentrations, and using Krogh's(13) observed values of oxygen diffusion constants for various tissues and the rate of oxygen consumption of his tissues, he was able to calculate the limiting thickness of the slice which would still permit the deepest cells to obtain oxygen   As he expressed it, $d = \sqrt{8 C o \frac{D}{A}}$, where d = the thickness of the slice of tissue in cms , Co = the external oxygen concentration, D = the diffusion constant for oxygen (expressed as the number of c c of O_2 passing through a tissue membrane of 1 sq cm surface and 1 cm thick when the difference of oxygen pressures on the two sides is 1 atmos), and A = the oxygen consumption of the tissue in c c O_2 per grm of tissue per min This may similarly be written $C = \frac{A d^2}{8 D}$ where C is the unknown oxygen pressure necessary for a tissue of thickness d

The nerves lying on a thermopile were laid parallel and in lateral contact, so they may be regarded as roughly forming a tissue slice with one small dimension   Eight similar nerves, cut at 2 0 cm length, weighed on the average 0 052 grm and, at specific gravity 1 06, occupied 05 c c The width is equal to eight times the depth, so d cm $\times$ 8d cm $\times$ 2 cm = 05 c c and d = 0 058 cm   In Warburgh's experiments, the tissue is exposed on both faces, whereas in these only one face is exposed to oxygen, so the effective thickness is twice as much, or d = 0 116 D, calculated from Krogh's data for fascia and corrected for temperature, is 1 1 $\times$ 10^{-5} (The true value for nerve is probably a bit higher because of the lipoids present) A, assuming the resting oxygen consumption above, is 0 00027 C, calculated from these data, is equal to 0 04 atmos oxygen pressure, that is, for the deepest nerve fibres to receive the oxygen necessary to maintain their resting metabolism, the surrounding gas must contain 4 p c oxygen For full activity nearly 20 p c would be needed! The general basis for this calculation has been thoroughly confirmed by Warburg experimentally, and its validity for nerve has also been shown(6)

It is not surprising, in view of these figures, that the nerve heat of activity falls readily in low oxygen containing media, and only reappears with 2 or 3 p c oxygen in the chamber The results of Fillie are not difficult to account for by the test used The nerve studied ran through an asphyxiating chamber, proximal to which it was stimulated and beyond

which it ended in its muscle The presence of a m i n i m a l muscle twitch was taken to indicate the presence of conductivity It is obvious that some fibres about the surface of a nerve will continue to receive oxygen long after the majority have been asphyxiated, and these would give a minimal contraction It is easy to estimate that in the nerves used here the thickness of the plate represents about 70 layers of individual fibres The bottom layer of fibres, therefore, receives oxygen at a concentration corresponding to 0 05 vol p c oxygen when the whole nerve is exposed to the critical 4 p c of oxygen, and at this concentration is able to function, so that fibres on the nerve surface should remain active in media containing this amount of oxygen, and probably even with considerably less The total heat and action current from such a few fibres would, of course, be immeasurable

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so gives less energy liberation (3) There is, however, the further possibility that even for widely spaced impulses the heat due to each is less in the absence of oxygen This would mean either (1) that all processes involved in conduction can be partially and equally inhibited—which by analogy with the action of narcotics may occur, or (2) that conduction depends on immediate processes for which oxygen is not essential and which produce but little heat, which are followed by secondary processes not essential to conduction but depending on oxygen and also producing heat The recovery heat of nerve comes at once to mind, but it has been shown that this fails in nitrogen no faster than the initial heat associated with conduction itself, so that if such an unessential heat producing reaction dependent on oxygen does exist it must accompany conduction itself and occur within a few sigma of the explosive liberation of energy associated with conduction It seems most unlikely that conduction should depend on some anaerobic reaction of little heat with an unessential explosive oxidation superimposed, and the whole followed by a large and prolonged anaerobic "recovery," and need not be further considered in the absence of direct evidence for it

The third factor mentioned, that as asphyxiation proceeds more and more fibres fail entirely to conduct, undoubtedly plays a rôle, and possibly the main one, in the fall of heat Evidence has been given that the deepest fibres are thrown out first in low concentrations of oxygen and presumably the uppermost ones fail last This statistical falling off of heat, however, gives no clue to what happens in each fibre, and it is of basic importance in the interpretation of activity to know how the resting nerve is affected Some suggestive evidence on this point has been given The resting heat production of nerve presumably does not fall at once to zero in nitrogen but gradually diminishes for some time Up to a certain stage of asphyxiation a fibre can regain full activity in oxygen, beyond this it is permanently inactive, as evidenced by the rate and completeness of recovery after different durations of asphyxia There is no question of the nerve requiring oxygen for its basal metabolism, nor is there doubt that when the reactions of maintenance are interfered with the nerve machinery must be disrupted and conduction fail Muscle, with a well-established anaerobic contractile mechanism, will also fail entirely to act when the medium is sufficiently depleted of oxygen (17) though with a little present it does as well as with much more (18) If this, however, be the sole cause of failure of nerve heat in absence of oxygen there is no reason to believe that conduction itself requires oxygen To borrow Lucas' analogy, telephone service would cease if the central exchange

were filled with nitrogen in place of air, but this does not prove that oxygen is required for the passage of messages along the wires There are many other means of stopping conduction—cold, pressure, narcotics, acids, etc —that give a fall of conductivity similar to that in nitrogen, which may act on the conductive mechanism or on the more basic machinery of cell survival Several investigators (15, 19) have in fact concluded that the failure of conduction in asphyxia is due solely to the accumulation of toxic metabolites, but this conclusion is open to criticism

The fact remains, however, that during activity a nerve uses more oxygen and produces more heat and carbon dioxide than when at rest The amount of heat produced agrees very well with the gas exchange observed on the assumption that the heat is practically all oxidative The fall of excitability, the slowed conduction, the tendency of action potential to give equal effects on charge and discharge, and other qualitative effects of oxygen lack, seem to indicate that it enters directly into the conduction processes On the other hand, though asphyxiation cuts out initial and recovery heat, there is no certainty that this is more than the result of the progressive failure of individual fibres due to interference with their basic metabolism

One possible way out of this antinomy has been mentioned in a preceding paper (2), and the time course of nerve heat failure in nitrogen falls in line with this After nitrogen has replaced air, the oxygen content of the nerves must have reached equilibrium, by diffusion or utilisation, within a few minutes Still the falling off of activity continues more or less slowly for two or three hours before it becomes immeasurable It is tempting to account for this by an internal reserve of oxygen or its equivalent "Intramolecular oxygen" has often been suggested in the past and as often denied. As applied specifically to nerve, Fröhlich (14), and more extensively Gottschalk in 1914 (19), argued in favour of an oxygen reserve from his observations that after being asphyxiated in nitrogen a nerve completely regained its conductivity (measured by a muscle twitch) in 2 to 3 min in oxygen but continued to show improved resistance to a subsequent asphyxia until it had been in oxygen for 8 min Subsequently (1921), from a somewhat too rigorous application of the mass action law, he concluded that all oxygen stored in a nerve would necessarily diffuse out after eight minutes in nitrogen, and that, therefore, the nerve has no oxygen store but conducts by an anaerobic mechanism. It need only be pointed out here that the assumption that all physically dissolved or chemically bound oxygen would diffuse out

of the nerve on changing its environment from oxygen to nitrogen in the same time that it took to enter when the reverse change was made is not valid

Living cells are normally poised at some characteristic oxidation reduction level, but if kept under anaerobic conditions this level steadily falls Cannan, Cohen and Clark (20) have shown that the electrode potential of a suspension of bacteria, yeast cells or liver, falls with time of anaerobiosis (= more reducing state) along an exponential curve, suggesting that a monomolecular reduction of some contained substance is taking place B E Holmes (21) has found 50 mgms of glutathione in 100 grm of white matter of rabbit's brain, and Keilin (22) reports the presence of cytochrome in brain tissue This amount of glutathione alone, if all oxidised at first (a doubtful assumption) and completely reduced by the tissue during anaerobiosis, would serve as an adequate oxidiser, or hydrogen acceptor, for 50 min metabolism of a nerve during activity and over 3 hours during rest Such figures serve at least to indicate how far an oxidation reserve might supply the extremely small energy requirements of active nerve A variation in the amount of such substances from fibre to fibre, the varying oxygen available at different depths, and different metabolic rates of various fibres, could account for the gradual decline of nerve heat, though the heat per fibre also probably undergoes a fall Similarly the delay of recovery in oxygen might be partly due to an excessive utilisation of oxygen by the outer fibres to restore their impoverished reserves, so that no oxygen would reach the deeper ones for some time

It is worth noting finally that, so far as all evidence hitherto obtained goes, the delayed heat of nerve is dependent only on the initial breakdown, no matter how the latter is varied the recovery heat follows This strongly confirms the previous suggestion that the delayed heat depends on a monomolecular reaction of some substance freed during the initial phase

Summary

1 Removal of oxygen from a nerve's environment causes

(a) A progressive fall and ultimate extinction in two or three hours of the initial heat of activity This is largely due to individual fibres becoming inactive, probably also in part to decreased heat per fibre

(b) A similar fall and extinction of the delayed heat The delayed heat continues to represent the same fraction of total heat and shows no tendency to fall more than the initial heat

(c) A progressive fall of resting heat production, ending with death of fibres if sufficiently continued

(d) A progressive fall and extinction of average action potential This does not parallel the fall of heat

2   All these return to or towards their original state when oxygen is readmitted to the system The action potential returns much more rapidly than the heat, which requires an hour or two

3   Practically complete asphyxiation of nerve can be obtained under the conditions of these experiments in nitrogen containing over 1 p c of oxygen

4   The capacity for activity of a nerve is definitely affected by previous activity within several minutes This appears to be a type of "fatigue"

5   The problem of oxygen diffusion into nerve and availability for its metabolism is considered

6   The above results are difficult to reconcile with a glycogen-lactic acid system, but do not disprove an oxidative mechanism for conduction, hydrogen acceptors or other oxidising bodies in nerve may permit oxidative activity for considerable time in the absence of oxygen

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THE RELATIONSHIP BETWEEN THE VOLUME OF THE HEART AND ITS OXYGEN USAGE

By A. HEMINGWAY and A. R FEE (*Junior 1851 Exhibition Scholar*)

(*From the Department of Physiology and Biochemistry, University College, London*)

RECENTLY Starling and Visscher[1], using the isolated heart-lung preparation, showed that a definite relation existed between the diastolic volume and oxygen consumption of the mammalian heart Their results supported the hypothesis that the factor controlling the ability of the heart to perform mechanical work is its diastolic volume, which is itself determined by the ratio between the total energy liberated and that actually utilised by the heart

In their experiments, Starling and Vischer (*loc cit*) measured only the ventricular volume of the heart Though the auricles do not constitute more than 10 p c of the cardiac mass it is desirable that they should be included in any measurements of heart volume The chief obstacle to including the auricles in such measurements is the difficulty of obtaining satisfactory records with the existing methods of cardiometry The introduction of a cardiometer possessing several new features enabled accurate measurements of the volume changes in the whole heart to be made and led us to repeat the experiments of Starling and Visscher

The method of measuring the oxygen consumption of the isolated heart-lung preparation, which will be described fully elsewhere, consists in introducing a known volume of oxygen into a closed air circuit connected with the lungs and measuring the time required for its consumption by means of a modified Krogh spirometer

The cardiometer commonly used at the present time for recording ventricular volume, as described by Henderson[2], has several serious drawbacks, the most important of which is that the rubber collar designed to fit into the auriculo-ventricular groove, if sufficiently tight to give accurate records of heart volumes, tends to constrict the pulmonary artery, the coronary blood flow, and even to hinder the passage of blood into the ventricles

The cardiometer used in these experiments (Figs 1, *a* and *b*) is a modification of that used by Hill and Barnard(3), who slit open the

a   Fig 1   *b*
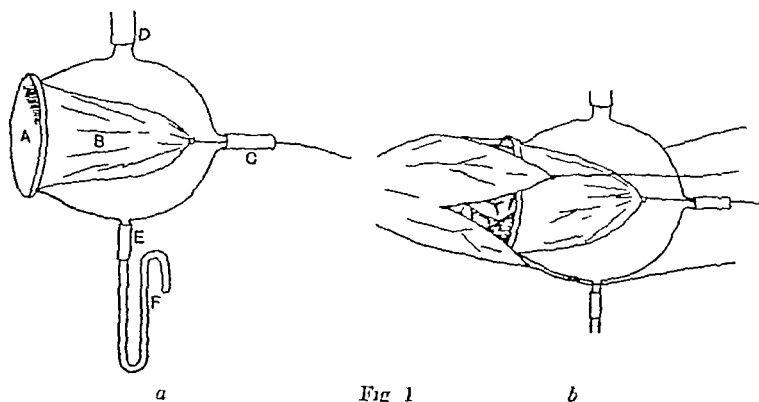
pericardium and thrust the heart through an aperture in a tennis ball, tying the perdicardium over the outside of the ball An obvious disadvantage of this method is the removal of the supporting pericardium Dilatation is entirely unrestricted and bruising and hæmorrhage of the heart wall may result from impact with the walls of the cardiometer To obviate these disadvantages, an oval glass shell has been designed (Fig 1, *a*) with a large, lipped aperture at one end (*A*) through which the heart is thrust A thin rubber sleeve (*B*) is attached to the margin of this aperture and hangs free within the shell The unattached end of the sleeve is closed and supported by a thread which passes through a small aperture and short length of rubber tubing (*C*) immediately opposite the large orifice for the heart This sleeve serves as a pericardium, and, after receiving the heart, may be adjusted and kept at the requisite degree of tension by clamping the thread within the rubber tubing Two more apertures are provided in the shell, one (*D*) for attachment to a piston recorder or tambour, and the other (*E*) for draining away any accumulation of pericardial fluid A few small holes should be made in the rubber pericardium to allow this fluid to drain away from the heart

The method of attachment is as follows After the heart has been exposed, a small opening is made in the pericardium over the apex The edges of this opening are then caught by three artery forceps and one posterior and two antero-lateral flaps made by slitting the pericardium between them until the opening is large enough to admit the mouth of the cardiometer After threads have been tied to these flaps the heart is slipped into the rubber sleeve and the pericardium is drawn over the

exterior of the cardiometer and tied into position about C (Fig 1 a) A ligature previously placed around the heart is then tied about the neck of the cardiometer Finally after attachment to a recording system the cardiometer is held in a suitable position with a universal clamp and the tension of the rubber sleeve adjusted

During the period of an experiment the accumulation of pericardial fluid within the cardiometer may simulate a dilatation of the heart by expelling air into the recording system To avoid this a resistance syphon F (Fig 1 a) is attached to E and filled with normal saline This will syphon off any fluid which may collect but will not interfere with the heart volume record

Results

It was found that the diastolic volume of the whole heart bears a direct linear relation to the oxygen consumption Undue dilatation of the heart, induced by excessive loading results in a disproportionate increase in the oxygen consumption but this discrepancy lies well outside the range of volumes found in a heart working under physiological conditions This is illustrated in Fig 2 in which the oxygen consumption of the heart is plotted against the diastolic volume the latter being calculated in the manner described by Starling and Visscher(1) The changes in volume were brought about by altering either the inflow or the peripheral resistance of the preparation, the order of these changes being indicated by the numbers attached to the points on the curve From this it can be seen that the oxygen consumption increases steadily with an increase in the diastolic volume Œdema of the lungs occurred during the last three determinations and its influence upon the results can readily be seen.

The above principle may be applied to the determination of the oxygen consumption of any isolated organ perfused by a heart-lung preparation, provided the diastolic volume oxygen consumption ratio of the heart and the diastolic volumes and oxygen consumptions of the whole preparation have been measured The oxygen consumption of the heart at any given time can be determined by direct measurement of its diastolic volume and reference to the graph constructed from the preliminary data By subtracting this value from the observed oxygen consumption of the whole preparation at that particular time, the oxygen consumption of the perfused organ is obtained

In a kidney perfusion experiment, the kidney was placed in the heart-lung circuit after several consistent values for the diastolic volume oxygen consumption ratio over different ranges of volume had been obtained

(as in Fig 2)   When the kidney was removed two and a half hours later the observed oxygen consumption of the heart was found to be 8 10 c c
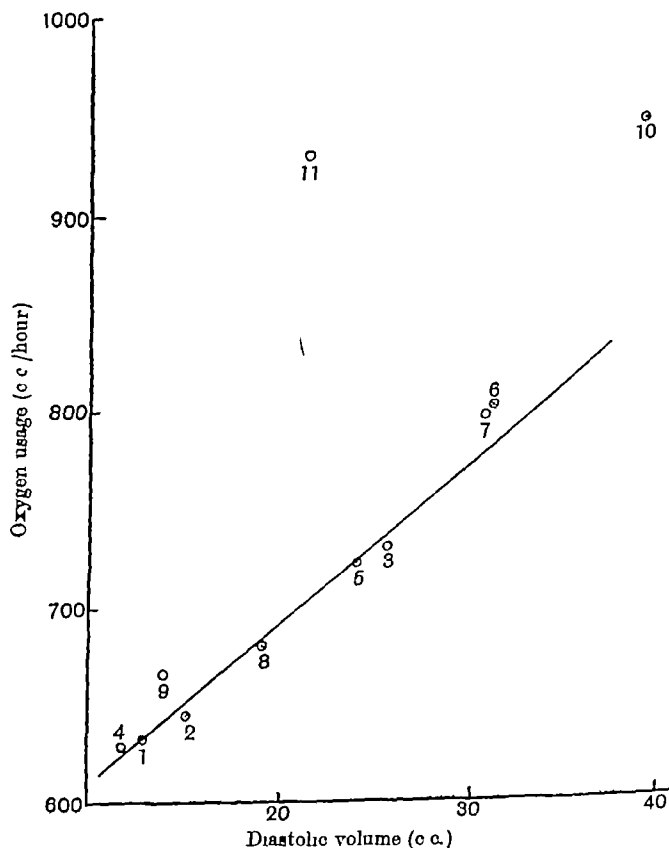
Fig 2

TABLE I

Reading	Time	Heart output c c /min	O₂ usage c c /hour	Heart diastolic volume x + c c.
1	1 00	425	632 45	13 00
2	1 12	700	645 48	15 20
3	1 30	285	732 00	25 50
4	1 45	430	629 52	12 90
5	2 00	—	724 68	24 00
6	2 15	—	805 20	31 00
7	2 25	—	797 88	30 55
8	2 38	270	680 76	19 10
9*	2 52	—	666 12	14 00
10*	3 09	215	951 60	38 00
11*	3 27	—	933 30	21 25

* Œdema

per mm The value corresponding to its diastolic volume, as given on the curve constructed from the preliminary data, was 7 92 c c per mm This agreement must be regarded as very satisfactory in view of the duration of the experiment and the amount of work that had been done by the heart in perfusing the kidney

SUMMARY

1 An improved type of cardiometer is described

2 The oxygen consumption of the whole heart bears a direct linear relationship to its diastolic volume

3 A method of determining the oxygen consumption of an isolated organ perfused by a heart-lung preparation is described

The subject of this research was the suggestion of Prof Starling His advice was always readily given and we deeply regret that he should not see the continuance of this work

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THE CIRCULATION OF BODY FLUIDS IN THE FROG
By EDWARD D CHURCHILL, FUSAKICHI NAKAZAWA
AND CECIL K DRINKER

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In the course of experiments dealing with the capillary circulation of the frog it was discovered that the fluid in the subcutaneous lymph spaces normally contains 0 29 to 2 17 p c of protein. As we were concerned with questions relative to capillary permeability it seemed of immediate importance to reach a clear understanding of this exchange of protein substances through the capillary walls. Fortunately the solution of the question has been aided by the recently published figures of directly measured capillary pressures in the frog mesentery by Landis[1], and by a volumetric estimation of the rate of total fluid exchange between the blood and tissues by Isayama[2]. To these facts we now add determinations of the protein content and the colloid osmotic pressure of the blood and lymph, and endeavour to combine these lines of evidence in a discussion of the physical and chemical forces which produce a constant and normal movement of the blood plasma with a large fraction of its proteins from the blood vessels into the lymphatic circulation

METHODS

The frogs used in the following determinations were chiefly *Rana temporaria*, although a few individuals of *R esculenta* are included. There appeared no significant difference between species or sexes

In the majority of cases the lymph was collected from the lymph sac which lies dorsal to the tendon of the gastrocnemius. In a few instances it was taken from the other subcutaneous lymph spaces, and in those specimens used for osmotic pressure determinations fluid from the peritoneal cavity was added to obtain the necessary volume. From pharmacological evidence Straub[3] considers that there is a free intercommunication of the lymph spaces with each other and with the peritoneal cavity, so that practically we have to do with one large lymph space

The analyses were carried out between March 9 and April 19 and therefore include individuals taken before the breeding season with

immature eggs in the ovaries, a number of individuals during the height
of the breeding season including some markedly œdematous copulating
males and specimens of both sexes which had apparently returned to a
normal condition after the completion of egg-laying It is not the purpose
of this paper to discuss the cause or significance of the spontaneous
œdema which occurs particularly in the males during the activity of the
breeding season, although measurements made on such individuals are
so specified in the tabulation The close correspondence between our
figures and those of the colloid osmotic pressure of the three plasma
specimens reported by White(4) for a different species at a different
season of the year make us feel that it is extremely unlikely that the
phenomena we are describing are peculiar to the spring breeding period
It is possible, however, that the absolute values given in our tables will
prove to be somewhat below figures which may be obtained for normally
feeding frogs that are not undergoing the depletion in body protein which
may attend the later stages of egg and sperm production

The protein quantitation was accomplished by the use of a Zeiss
Eintauch-Refraktometer (Pulfrich), using Prism 1 The necessary correc
tion factor for the non-protein constituents of the plasma was obtained
from the ultra-filtrate of a specimen of frog lymph The scale reading of
this ultra-filtrate was 18 4 which indicates an nD 1 33450 This corre-
sponds very accurately with the nD of Ringer's solution made with the
composition given by Clark(5) The nD of 1 p c of frog-lymph protein
was found to be 0 00240 by the analysis of a specimen of lymph with
a known refractometer scale reading[1]

The osmotic pressure determinations were carried out according to
the method of Krogh(6) Both the blood and lymph specimens were
allowed to clot and the centrifuged serum employed in the analyses

RESULTS

The determinations are expressed in the form of the three following
tables

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[1] 3 c.c. of lymph weighing 3 025 grm. were diluted to a volume of 50 c.c with water
A 20 c.c. portion of this dilution was heated and the nitrogen content of the resulting
coagulum determined by Kjeldahl analysis was 0 0025 grm. Assuming that the coagulum
represents the total protein and using the factor 6·45 for the conversion of weight nitrogen
to weight protein, 100 c.c of lymph (100 83 grm.) contains 1 34 grm. protein. The per cent.
of protein by weight is therefore 1 33

In using this value it is assumed that the heat coagulum represents the total protein
content of the specimen. A second 20 c c portion of the same dilution contained 0 00334
grm. total nitrogen as determined by Kjeldahl analysis.

TABLE I

Frequency distribution of the percentages of protein in the fluid
from the lymph sacs of 60 normal frogs.

p c	No of individuals
0 29 to 0 69	9
0 74 to 1 35	38
1 38 to 2 17	13

TABLE II

Percentages of protein in the fluid from the lymph sacs of seven frogs with the marked
œdema which accompanies the mating season.

p c.		p c
Frog 1	0 60	Frog 5　1 65
2	1 07	6　1 73
3	1 38	7　1 78
4	1 57	

TABLE III

Colloid osmotic pressures of blood and lymph
(Including determinations by Krogh 1922 and White 1924)

		Blood			Lymph		
	Descrip ion	Osmotic press mm water	Protein p c.	$\frac{O P}{prot}$ p c.	Osmotic press mm water	Protein p c	$\frac{O P}{prot}$ p c.
1	Normal male	69 0	2 87	24 0	37 0	1 50	24 6
2	Normal male	68 0	2 49	27 3	28 0	1 22	22 9
3	Normal male*	134 0	4 29	31 2	77 0	2 17	35 4
4	Emaciated female	15 0	1 39	10 8	5 0	0 43	11 6
5	Mixed 4 frogs	69 0	—	—	—	—	—
6	Œdematous male	54 5	2 01	27 0	28 5	1 07	26·6
7	Œdematous male	66 0	2 61	25 2	47 0	1 77	26·5
8	Œdematous male	76 0	2 80	27 1	37 0	1 37	25·0
9	Mixed 4 œdematous males	86 0	3 27	26 3	77 0	1 60	48 1
	Krogh, 1922						
Frog's blood hirudinised		55 0	2 1	26 0	—	—	—
"		60 0	1 5	40·0	—	—	—
	White 1924 (oxalated)　mm plasma						
Frog 1		96·0	2 4	40 0	—	—	—
Frog 2		98 0	2 52	39 0	—	—	—
Frog 3		115 0	2 8	41 0	—	—	—

* It is to be noted that of the 60 frogs included in Table I this animal showed the
highest percentage of protein in the lymph

DISCUSSION

In presenting his figures for the osmotic pressure of the blood colloids
of the frog, White[4] points out the forces which might bring about
filtration through the glomerular capillaries The subsequent measure-

ments reported by Hayman(7) which show glomerular capillary pressures higher than the ones of Hill which are cited by White add further evidence that this filtration may occur   Landis(1) by a method of direct measurement has found that the average pressure in the arteriolar capillaries of the frog mesentery is 14 5 cm of water, and that of the venous capillaries is 10 0 cm of water   Using the figures of White this author points out that capillary pressure may be above or below the osmotic pressure of the plasma colloids throughout the whole length of any capillary in successive moments   As our figures are lower than those given by White, they make it more safe to conclude that there exists a filtration of fluid throughout the systemic system to the extent that it may be assumed that the capillary pressures of the mesentery hold for other regions of the body   In making such an assumption it is important to recall the fact that the frog possesses two portal systems, that of the liver and that of the kidney   Both of these organs impose the obstruction of a second capillary bed in corresponding portions of the venous system The liver receives the venous blood of the intestinal tract including the rectum and bladder as well as blood from the Bulbus cordis, from the abdominal wall and a part of that of the hind legs   The kidney receives the remainder and larger fraction of the blood from the hind limbs, as well as blood from the dorsal part of the trunk and the rump and in the female from the oviduct (8)   It is almost certain that the existence of a secondary capillary bed must act as an important factor in the determination of the pressure gradient in the vessels of the primary circuit

Another factor acting to increase the volume of filtration is the presence of 0 29 to 2 17 p c of protein in the fluid of the lymph spaces From this finding we must infer a degree of permeability to protein on the part of the walls of adjacent capillaries which is entirely unlike that commonly assumed to exist in mammals except in certain tissues such as the liver   It is our impression that this increased permeability is a characteristic of the vessels of the skin of the frog   The filtration of fluid will naturally be increased wherever the effectiveness of the already low intravascular colloid osmotic pressure is reduced by this permeability of the capillary wall to protein   Thus the average osmotic pressure of the colloidal proteins of the lymph has been found to be 42 mm of water and the difference between this value and that of the intravascular 71 of the blood colloids is but 29 mm of water representing the effective colloid osmotic pressure tending to hold fluid within the capillary Opposed to this, at least in the mesentery, is a capillary pressure averaging 122 mm. of water

It is of interest therefore to find that Isayama[2] working from an entirely different point of view has estimated that in the toad a volume of fluid equal to that of the total blood plasma passes from the vessels into the tissues and is collected by the lymphatic system at least fifty times in twenty-four hours This writer estimated changes in blood volume by counting the erythrocytes and determined the amount of blood volume diminution during the first ten minutes following the destruction of the lymph hearts

The physiological significance of the actively beating lymph hearts of the frog at once becomes obvious if we consider that we are dealing with an animal which is carrying on a general filtration of fluid from its systemic circulation, and in addition possesses at least a large capillary area which is freely permeable to the protein constituents of the blood plasma

SUMMARY

The protein content of normal frog lymph varies from 0 29 to 2 17 p c , the usual value being 1 00 p c The average osmotic pressure of the colloids of the blood serum of nine frogs was 71 mm of water In the same individuals the average colloid osmotic pressure of the lymph was 42 mm of water

These values considered in the light of known capillary pressures show physical and chemical forces acting to produce a normal movement of blood plasma including a fraction of its protein from the blood vessels into the lymphatic system This is consistent with published volumetric estimates of the rate of fluid exchange in the frog

Such a concept is of the utmost importance in experimentation involving the use of the frog for the study of capillary permeability

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THE INFLUENCE OF VARIOUS SUGARS ON THE RESPIRATORY QUOTIENT   A contribution to the significance of the R Q

By E P CATHCART and J MARKOWITZ

(From the Institute of Physiology, Glasgow University)

ALTHOUGH the respiratory quotient has been determined in many investigations, since the special relationship of the carbon dioxide output to oxygen consumption was first recognised by Lavoisier, established on a firm basis by the work of Regnault and Reiset and definitely referred to by Pflüger as the Respiratory Quotient, there seems to be a tendency in many quarters to look on this ratio as a definite unit of unequivocal significance   The fact is only too frequently not appreciated or, if appreciated subconsciously, is ignored in practice that the CO_2/O_2 ratio represents not a single, relatively simple, physiological phenomenon but is the sum of an infinitely large, relatively unknown, series of phenomena   To-day, with the more frequent determinations of the respiratory quotient due to the interest aroused in the chemical changes which take place during the contraction of muscle and, even more predominantly, by the mass of work on carbohydrate metabolism incited by the discovery of insulin, it is all the more necessary that there be a proper appreciation of the true significance of this quotient   It cannot, we think, be held to misstate the present casual interpretation to say that judging from published evidence of many workers the CO_2/O_2 ratio is believed to be sound evidence of the nature of the *combustion* processes taking place in the organism at the time of the determination   It is very obvious that, unless the experiment be carried on for a considerable period by some chamber method, this simple explanation can never be valid inasmuch as the CO_2 cannot possibly be wholly a derivative of the particular O_2 taken in   Naturally the longer the duration of the experiment the more probable it is that the CO_2 output is directly related to the O_2 consumed   This obvious and simple fallacy, as well as the other, that the output of CO_2 may be due in large measure to excessive pumping out such as occurs in forced breathing and even in other less obvious respiratory disturbances, has of course been apprehended by many

workers But, on the other hand, many of those who both directly and indirectly infer that the ratio is indicative of the nature of the material combusted entirely overlook the equally obvious fact that the CO_2 level may alter not from an actual combustion of food-stuffs in the body but merely by metabolic transformation (*a*) with a marked increase in O_2 consumption as in the conversion of fat or protein to carbohydrate, and (*b*) without a corresponding utilisation of O_2 as in the conversion of sugar to fat

The question of the significance of the R Q takes us however into much more complicated phenomena than the relatively simple series, on paper at least, of chemical changes involved in the combustion of the various food-stuffs The difficulties involved in the utilisation of this ratio have been rather implicitly than explicitly acknowledged by really skilled workers like Zuntz and others, when, in order to simplify their calculations, they deal with the non-protein quotients, *i e* they either state that the protein element in the organism has undergone so little change during the limited period of the experiment that it can be ignored, or else due allowance is made and the residual CO_2/O_2 is held to represent the combustion values of the fat and carbohydrate But when considered in this simple and convenient fashion the assumption has to be made that we can speak of clean, straightforward combustion, that these non-protein fuels are stoked, so to speak, on to the cellular furnaces and are burnt in the same effective manner as takes place in the bomb calorimeter This would no doubt be a perfectly good conclusion if we were dealing with a static mechanism But if any one point is clear in our physiology it is that whatever the ultimate "mechanism" of living tissue may prove to be it is certainly not static We come back necessarily to the old quarrel between Bonn and Munich Is it necessary or not that the material ingested become an integral part of the living protoplasm before it is utilised? No unequivocal answer can be given to this question but we are of the opinion, contrary to many workers, that the balance of evidence is in favour of the view that the material to be utilised must become in some way an integral part of the protoplasm or living matter before it can undergo either anabolism or catabolism If this be so it is folly to speak of the disturbances of CO_2 output and O_2 utilisation, as manifested in the R Q , as evidences solely of processes of combustion Of course it is true, there being abundance of evidence in support of the statement, that if the intake of material is predominantly fat or carbohydrate there occur very definite and, on the whole, very constant changes in the character of the R Q The process of maintaining cellular equili-

brium is brought about however not merely by burning off the excess
of any one food-stuff given but by an infinitely finer series of metabolic
changes which lead to transmutation rather than destruction of the
ingested materials The whole trend of modern chemical physiology is
to show, that despite the apparent dissimilarity of the proximate
principles consumed as food, within the living cell there is a chemical
lability The old hard and fast distinctions and presumed fixity of
structure and entity of the various components are no longer adhered to
Further there is good evidence which points to the fact that these various
components cannot be utilised independently In other words although
the ultimate end may be combustion, we cannot now conceive that the
sole function of the O_2 absorbed is to bring about the *combustion* in a
direct fashion. Armsby(1) put the problem in an interesting way when
he wrote, "The metabolism of matter and energy in the body might be
compared to the exchange of water in a mill pond The water in the
pond may represent the materials of the body itself, while the water
running in at the upper end represents the supply of matter and energy
in the food, and that going down the flume to the millwheel the meta-
bolism required for the production of physiological work.   The water
flowing into the pond does not immediately turn the wheel, but becomes
part of the pond and loses its identity Part of it may be drawn into
the main current and enter the flume comparatively soon while another
part may remain in the pond for a long time

That this conception of Armsby s is no mere simile is shown very
clearly for the most hall marked of the three principal constituents of
food namely the proteins Repeated superimposition experiments have
shown that ingested protein is not necessarily utilised forthwith but that
it may, according to its nature, be retained in part for three four or
five days after its ingestion. Perhaps it is more accurate to say that
nitrogen ingested in the form of protein does not wholly reappear in
equivalent amount until after the expiry of the times stated. We have
no warrant of course for asserting that the excess nitrogen which does
appear is the actual nitrogen which was ingested. The experiments,
too, which have been carried out on the rates at which nitrogen and
sulphur are excreted, after their ingestion in the form of protein, go to
show that very special changes occur during the course of metabolism
which cannot possibly be explained on any simple combustion hypo-
thesis

Owing possibly to their simpler structure and especially to the fact
that they neither contain nor give rise (normally) to any identifiable end

products excreted by way of the urine, we cannot make such definite assertions in the case of carbohydrates and fats as we seem entitled to do in the case of protein

When all is said and done, to most workers, *the* fact which firmly establishes the value and significance of the R Q is that the data obtained by direct and indirect calorimetry agree so well, that, as there can be no gainsaying the direct method of measurement of heat loss, the indirect method, which is based on caloric values of a litre of oxygen varying with the R Q, must of necessity be valid But it must be remembered in this connection that in order to obtain reasonably close agreement between direct and indirect calorimetry the determination of the R Q need be only approximately accurate, as the caloric value for a litre of O_2 when the R Q is 0 71 is only from 5 to 7 p c less (according to the data used) than when the R Q is unity[1]

Murlin and Lusk(2), in a beautifully executed series of experiments on a dog, found, for example, that in nineteen out of thirty-two experiments the difference between indirect and direct calorimetry was less than 2 p c and in two of the experiments it was less than 1 p c This would seem to clench the matter and settle for all time any discussion as regards the validity of calculating heat loss from an R Q basis Du Bois and Gephart(3) showed also, on the basis of their investigations on human subjects, that there is remarkable agreement between the two methods not merely when long periods are considered but when periods as short as one hour are taken In a total measurement of nearly 5000 calories from seven subjects the two methods came within 0 17 p c of each other and on a larger number of subjects, both healthy and diseased, with a total measurement of 27,632 calories the direct calorimetry value was 1 62 p c inferior to the indirect

We do not wish to cast any doubts on the validity of the calculations of indirect calorimetry when the experiments extend over several hours, indeed, we are whole hearted believers in the method under these conditions We even believe that when conditions in the body are stabilised (as they presumably are in the post-absorptive condition when basal metabolic rates are commonly determined) agreement between indirect and direct calorimetry will be close Thus if the series of basal metabolic experiments given by Murlin and Lusk be statistically analysed, it will be found that although the standard deviation of the data obtained by the indirect method is some 20 p c greater than that

[1] Further the non protein R Q of 0 71 assumes that fat and fat alone is being burnt, an assumption which in the light of modern knowledge is perfectly inconceivable

obtained from the data of the direct method, yet the differences between the two means can be regarded (accepting the current statistical criterion) as being insignificant

But where metabolism is actively proceeding in the organism, as may occur in short experiments following food, this close agreement cannot be expected If the R Q were merely the index of combustion, which it is commonly assumed to be, agreement should be as close under these conditions as in the post-absorptive state If experiments carried out when the metabolism is active be continued sufficiently long however conditions again become stabilised so that the agreement between the sum of hourly periods by the two methods may be very close and yet, especially in the early periods when metabolism is active, the agreement in the individual short periods may be less good We have analysed the experiments in which glucose was given in the Murlin and Lusk series Admittedly the number of experiments is not very large, only 6, giving 23 periods in all We find that here again when the total period, i e the sum of all the values, was considered, that the difference existing between the data obtained from the indirect and direct methods may be regarded as being statistically non-significant Again the standard deviation of the direct data is lower than that of the indirect data The fact that in each case the standard deviation was lower with the direct means simply that there is an additional, improperly assessed, factor involved in the calculation by the indirect method When going over the data we noted that in all the experiments (this holds good for many other experiments besides those cited now) the indirect measurement figures were invariably higher than those of the direct during the first periods, i e the periods beginning, as a rule, about 45 minutes after giving the sugar and extending over the hour during which the metabolism is presumably most active It is questionable, of course, if statistical treatment of such a short series of figures is justifiable, nevertheless we tested the data by the same methods as employed above and found that the difference found between the indirect and direct methods was statistically significant One deduces then that some factor or factors entering into the calculation of the heat output by the indirect method has not a uniform or constant value The methods by which both CO_2 and O_2 are determined, especially in a laboratory like Lusk's, are as good as they can be made but, on the other hand, the calculation of the oxygen value into heat units is based on the assumption that combustion, and combustion alone, is taking place and these values are dependent on the validity of the R Q In other words the non-agree-

ment found may be ascribed to the faulty interpretation of a perfectly determined R Q

Further evidence that it is the R Q which is the determining factor in this discrepancy, which exists between the two determinations, is afforded by a further statistical study, not only of the glucose experiments but also of those in which fat was given in the same series of Murlin and Lusk, in which the correlation ratio between the R Q and the differences found between the data of the first and second periods obtained by indirect and direct calorimetry is determined   A positive correlation of 0 41 ± 0 112 is found, which indicates clearly that the non-agreement is in large part due to some factor varying directly with the R Q   In all probability this variable factor is the caloric value assigned to the oxygen   We therefore reach the conclusion that the R Q when metabolism is proceeding at an active rate must indicate more than mere combustion, that changes other than complete conversion of the non-protein substances into CO_2 and H_2O are also taking place

Benedict and Carpenter[4] in their study of the stimulating effect of nutrients state very definitely (p 173), that the agreement between the results obtained by indirect and direct calorimetry was in many instances extremely unsatisfactory, and they come to the conclusion that direct and indirect calorimetry may not necessarily agree under abnormal conditions such as occur when excessive amounts of carbohydrate are ingested (*e g* 100 grm glucose)   It will be noted that they specifically refer to carbohydrates, the food-stuffs which on ingestion bring about most readily considerable differences in the level of the R Q

In the course of an investigation, which had a somewhat different aim, we found that the effect of the administration of different sugars produced very considerable differences in the R Q curve   When we made our observations we were not aware that very similar experiments had previously been made by Higgins[5] and by Benedict and Carpenter[4]

Method Our subject, a young adult male, weighing 58 kilos, came to the laboratory in the morning in the post-absorptive condition   After a complete rest on a comfortable bed for at least 30 minutes his resting basal metabolism was determined, using the Douglas-Haldane method The subject then swallowed 50 grm of the sugar selected (47 grm in the case of the disaccharides) dissolved in about 200 c c water Air samples were then collected at half-hour intervals, usually for two hours Where blood sugars are reported, the blood was taken from a finger puncture immediately after the collection of the air sample The analysis of the

expired air was always done in duplicate All our results are strictly comparable since they were obtained under identical conditions The figures illustrating these results are drawn to the same scale All sugars used were the purest procurable

Results obtained As shown in the following table (which is a composite one, obtained by averaging the results from the various experiments, the individual values being given in the various graphs) the

TABLE I

Periods	Basal (2)*		Glucose (5)*		Lævulose (3)*		Galactose (1)*	
	R Q	O$_2$ c c	R Q	O$_2$ c.c	R Q	O$_2$ c.c.	R Q	O$_2$ c.c
Basal	820	207	855	198	809	208	777	211
After sugar								
End of 1st half hour	833	214	860	222	1 025	216	1 002	229
End of 2nd half hour	810	212	927	222	1 040	216	989	224
End of 3rd half hour	805	213	916	208	907	227	909	230
End of 4th half hour	—	—	883	211	—	—	—	—

Periods	Cane sugar (3)*		Maltose (1)*		Lactose (2)*		Dihydroxy acetone (2)*	
	R.Q	O$_2$ c c	R Q	O$_2$ c c	R Q	O$_2$ c c	R Q	O$_2$ c.c
Basal	852	204	766	197	846	201	807	204
After sugar								
End of 1st half hour	1 002	210	801	231	841	207	1 145	238
End of 2nd half hour	997	214	917	234	899	207	882	226
End of 3rd half hour	881	214	877	227	915	210	839	208
End of 4th half hour	832	217	—	—	—	—	851	200

* These numbers give the number of experiments done with each sugar, the average of the results being given in the column below

different sugars have very different effects on the R Q The normal course of the R Q under strictly basal conditions in two experiments, which were carried out on the same subject in an exactly similar fashion to the other experiments, is also recorded.

As the result of the ingestion of glucose the R Q either remains practically unchanged, or may even fall slightly at the end of the first half-hour period The maximum rise takes place at the end of the second period and is followed by a definite fall As regards the oxygen consumption values, a very definite rise in the utilisation occurs at the end of the first half hour, which is equalled by that at the *end of the* second period The consumption remains elevated during the two remaining periods The other two monosaccharides bring about similar but greater alterations in the course of the R Q despite the fact that one is an aldose and the other a ketose A very sharp rise is found at the end of the first period, a rise which, in the case of lævulose, is continued in the second period but which declines somewhat in the case
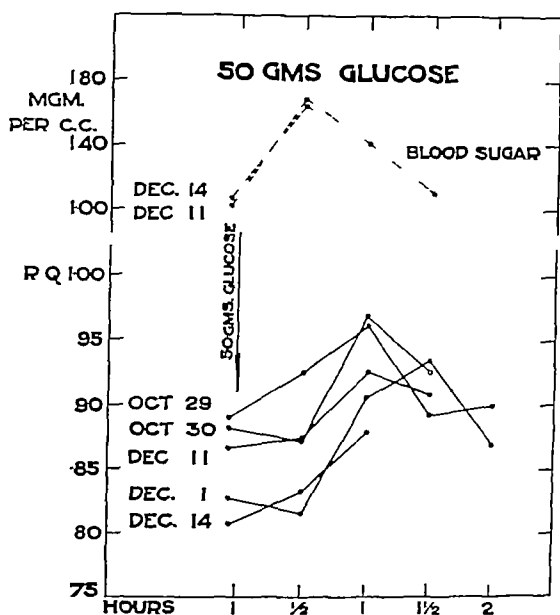
Fig 1
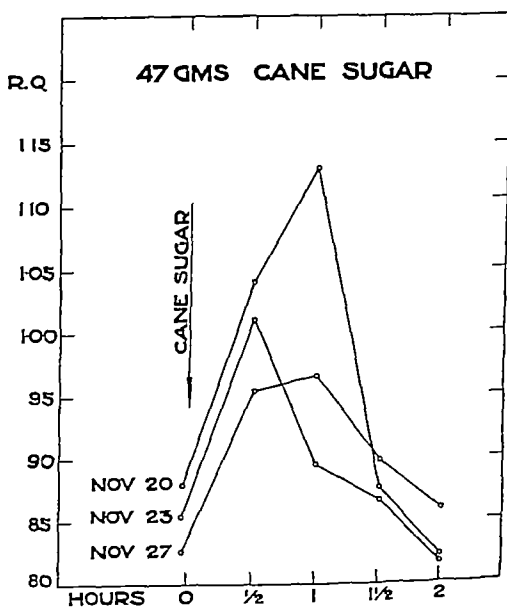
Fig 2

of galactose It continues elevated in both in the third periods With both sugars there is some increase in the oxygen consumption, but in
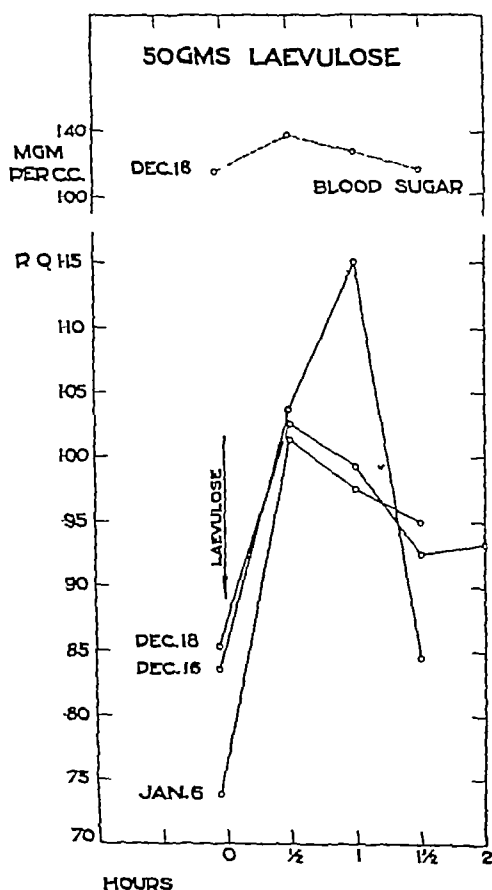
Fig 3

neither is it so great as is the case with glucose in the first and second periods but in the third period in both it definitely exceeds the glucose utilisation. As regards the disaccharides cane sugar undoubtedly produces the most definite changes in the gaseous exchange There is a very definite rise in the R Q at the end of the first half hour which is succeeded by a slow but unmistakeable fall The increase, however, in the oxygen utilisation is not a definite feature In the case of maltose the result obtained resembles somewhat the effect of glucose except that the rise in R Q at the end of the first half hour is greater, possibly due to
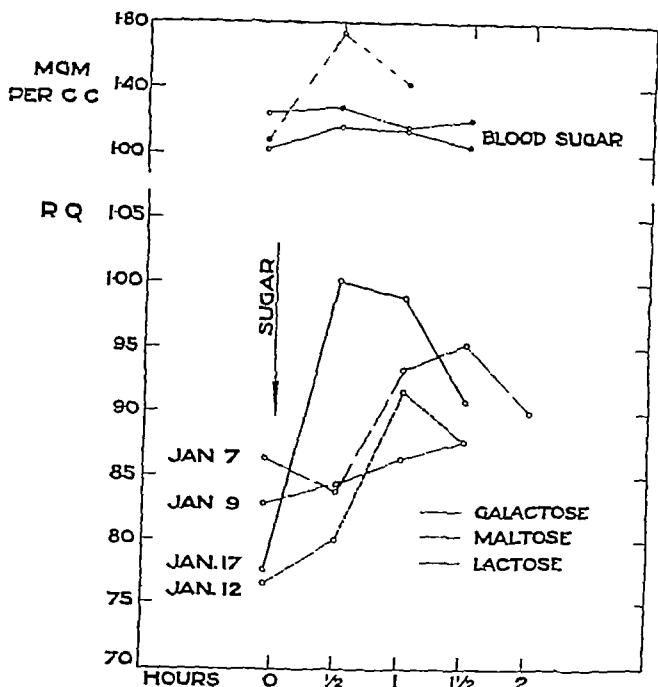
Fig 4
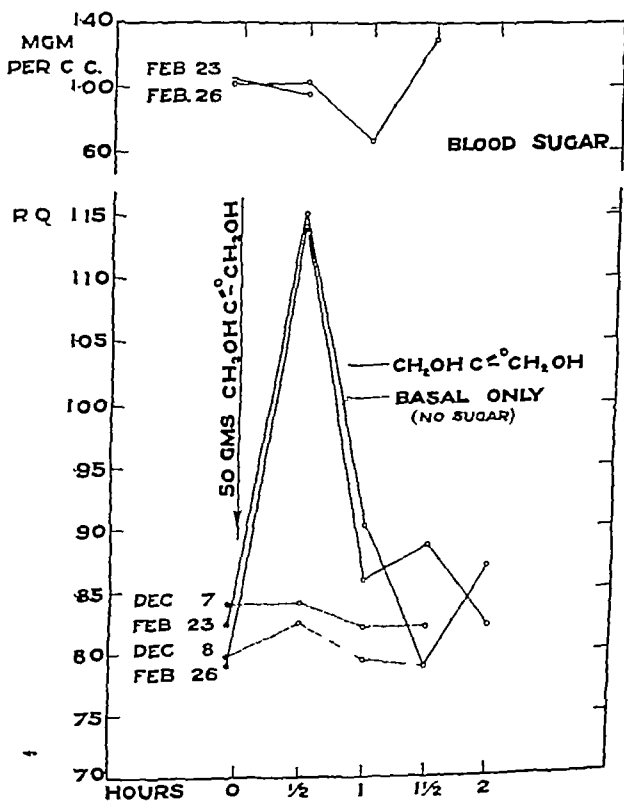
Fig 5

the low initial value and that the consumption of oxygen is quite definitely greater Lactose on the other hand seems to exert its action much more slowly, the maximum R Q not being obtained until the end of the third half hour The oxygen utilisation as the result of the ingestion of this sugar undergoes but little change, even when the R Q reaches its maximum the consumption is not greatly enhanced It is probable that this sugar did not undergo any marked hydrolysis in the intestine as in both experiments it caused some diarrhœa In the case of dihydroxyacetone there is a remarkably sharp rise in the R Q found at the end of the first period, which is followed by a fall almost as sudden There is a very definite rise more marked than with any other sugar except maltose, in the consumption of oxygen

We also investigated the influence of the ingestion of these various carbohydrates on the course of the blood sugar (Maclean's method) Following the ingestion of glucose and maltose the blood sugar shows a smart rise to a maximum value in half an hour In the case of lævulose there is only a slight rise in the copper reducing power of the blood and this is even less marked with lactose, galactose and dihydroxyacetone, the last substance, indeed, actually inducing hypoglycæmia

Our results agree with those of Higgins(5) in that lævulose, galactose and sucrose caused a sharper and more marked rise in the R Q than did glucose Tögel, Brezina and Durig(6) found that the R Q after the administration of lævulose rose more promptly and to a higher level than that following glucose Benedict and Carpenter(4) also noted that the P Q after the ingestion of lævulose and sucrose rose higher than that following glucose As regards the influence of dihydroxyacetone Himwich *et al* (7) found that the P Q in a dog following the injection of this substance was very much higher than that after the administration of corresponding amounts of glucose Mason(8) obtained identical results in the human subject

Discussion of results It is an extraordinary observation that, as judged by the R Q , the tissues combust with much greater facility than the alpha-beta glucose those monosaccharides which are normally absent from the blood. If this were so, it may be noted, it would be quite contrary to the results which have been obtained with amino acids(8) There is however no *direct* evidence that the organism can oxidise these monosaccharides without first converting them into glucose and the R Q in these experiments cannot be interpreted as indicating the preferential combustion of monosaccharides foreign to the blood stream. We believe the explanation of the apparent anomaly is given by the consideration

of the fate of dihydroxyacetone in the animal body This substance cannot be oxidised as such, but must first be converted into glucose by the liver(9) Thus when administered to depancreatised dogs receiving neither food nor insulin it was excreted quantitatively as glucose in the urine, most of it within a few hours During this time the R Q , as a rule, fell to about 0 67(10) It exerted no influence on the hypoglycæmic convulsions of hepatectomised dogs and when injected into nephrec tomised, surgically hepatectomised, preparations it was recovered unchanged in the skeletal muscles several hours later

What is true of dihydroxyacetone is probably more or less true of the other sugars Lævulose, for example, is excreted quantitatively in the urine of depancreatised dogs as glucose, the R Q being unaffected by the administration When fed to a liverless dog moribund from hypoglycæmia lævulose neither brings about recovery nor does it, unlike glucose or *d*-sodium lactate, lead to a deposit of muscle glycogen(11)

A plausible explanation, therefore, for the great rise in R Q following the giving of dihydroxyacetone, lævulose and galactose is that these subtances, being abnormal constituents of the blood, are rapidly removed from the circulation by the liver The absence of alimentary hyperglycæmia is good evidence in support of this view The transformation of these substances into glucose raises the tension of carbohydrate in the liver to a degree sufficient, not only to stop glyconeogenesis out of fat and protein but also to bring about a considerable synthesis of fat

It is not easy to accept this explanation as its experimental verifica tion is beset with many difficulties But whether the hypothesis be correct or not, since there is no evidence that the tissues can oxidise dihydroxyacetone directly the influence of this substance on the R Q can only mean that this quotient is at least not a combustion quotient It is probable that this response, moreover, is not an anomalous or exceptional one Thus when a mixture of fat and carbohydrate is completely burned outside the body the relative proportion of these is obviously reflected in the R Q Based upon this proposition tables have been constructed which purport to indicate from the R Q the exact proportions of fat and carbohydrate undergoing complete oxidation in the animal body Such a calculation is fundamentally unsound since, as already suggested, it makes the very improbable assumption that the phenomena of metabolism occur in a tripartite system, in which fat, protein and carbohydrate are being oxidised synchronously and at the same time independently of each other, just as though they were being oxidised *in vitro* The R Q assumes quite a different significance in the

simultaneous presence of extensive syntheses or decompositions The organism is always manifesting these changes, either when fed or fasting During the course of the day enough food is ingested to provide sustenance for the night period Let us consider the relatively simple case where a diet (just sufficient to cover the energy requirements) consists of protein and carbohydrate exclusively, the protein supplying 15 p c of the total energy Such a diet has of course a non-protein R Q of unity An organism subsisting on this diet for several days should have an R Q of about unity before breakfast, if none of the previously ingested carbohydrate be stored as fat Benedict and Higgins[12] fed a number of subjects for four days on a diet containing 600 grm of carbohydrate, 100 grm protein and 24 grm fat, equivalent to 3050 cals The non-protein R Q of such a diet is 0 97 The basal R Q however in the post-absorptive condition averaged only 0 87 Although the energy intake in these experiments was well in excess of the requirements, the fact that the fasting R Q was no higher than 0 87 can only mean that during the day much of the stored glycogen was transformed into fat and that in the "unfed" period the newly synthesised fat was contributing to the energy requirement, possibly by oxidation through a carbohydrate stage It is impossible in our opinion to escape this conclusion To account for the comparatively low post-absorptive R Q the non-protein R Q during the day would require to show a compensatory rise to over unity These considerations render inevitable the conclusion that the phenomena of anabolism and catabolism are in dynamic equilibrium, anabolism, with its attendant syntheses, predominating during the day and catabolism, with its attendant decompositions, during the night When the body weight is constant, the average R Q taken over a period of 24 hours must reflect accurately the nature of the diet, since the error incurred by too high an R Q during the day would be neutralised by too low an R Q during the night

On an ordinary mixed diet the R Q during the day does not exceed unity as there is enough fat in the diet to keep the R Q down The post-absorptive R Q is however lower than that found during the course of the day The same criticism, therefore, is valid for any diet in which the food is ingested in three or four meals during the day In order to obtain a true picture of metabolism from an isolated determination of the R Q the food intake would have to be spread out over the 24 hours, so that, for example, the individual ingested every half hour $\frac{1}{48}$th of a mixed sample of the daily ration

The fact that the non-protein P Q during prolonged starvation lies

in the neighbourhood of 0 71 is, we believe, evidence of the correctness of this reasoning   In this condition the glycogen content of (*a*) the liver, and (*b*) the muscles has become stabilised   As the glycogen breaks down respectively (1) to maintain the normal sugar concentration of the blood, and (2) to supply energy for muscular activity and the maintenance of body temperature, fats and proteins break down to replace the glycogen utilised, the process being in a state of dynamic equilibrium   It is for this reason that prolonged starvation will not render a rabbit glycogen free, even when accompanied by a preliminary "deglycogenation" by means of strychnine and cold (13)   The glycogen re-accumulates as soon as these latter influences have passed away   The glycogen which is always present in the tissues in starvation is therefore a dynamic and not a static substance, as is sometimes erroneously supposed   An R Q of 0 71 in starvation indicates that although the organism utilises glycogen for cardiac contraction, for respiratory movements and for a certain amount of voluntary muscular movement, the replacement of this glycogen from fat and protein is a steady process, the algebraic sum of the R Q 's of these transformations being 0 71   Even a single estimation of the R Q in starvation is probably an accurate index of the type of metabolism, since the organism is utilising its body fat and protein not in three meals a day but more or less continuously

Many investigators have studied the R Q during muscular exercise in order to determine the nature of the fuel combusted by contracting muscle, without considering the dynamic nature of muscle glycogen That there has been considerable disagreement until recently is not surprising in view of the many variables requiring control   In short bouts of muscular exercise in man the R Q in excess of the basal during exercise plus recovery works out exactly to unity (14)   It may be assumed, therefore, that the muscles of an intact mammal use carbohydrate as their fuel, just as is the case in the isolated gastrocnemius of the frog When this method of calculation is applied for longer periods of exercise the R Q , however, works out to less than unity (15)   This, we believe, indicates that the depletion of glycogen during prolonged muscle work brings about a compensatory breakdown of fat to replace the glycogen which is being used up

Quite apart from mere "auspumpen," the R Q , as it is usually calculated, may obviously be very variable, depending on the severity of the exercise and the "condition" of the subject   The mildest type of muscular activity possible occurs when a fasting individual reclines on a couch   Although the heart and respiratory muscles under these

conditions must be utilising carbohydrate in appreciable quantities the R Q is approximately 0 71   Fat is evidently replacing glycogen as rapidly as the latter is being used up   When a fasting dog was made to walk on a treadmill at the rate of 3 miles an hour for 3 hours the R Q was 0 71 (16)   Under these conditions, again, glyconeogenesis out of fat exactly balances glycogen breakdown in the muscles   When the muscular exercise becomes more severe the R Q may or may not rise, depending on the ability of glyconeogenesis to counterbalance glycogenolysis   In the case of superbly trained athletes the R Q in the post-absorptive condition during short bouts of maximal effort (rowing) was often 0·7 (17)   Here again glyconeogenesis and glycogen breakdown are in equilibrium   When however the muscular exercise is prolonged as well as severe the R Q rises often to nearly unity (18)   The extent of the rise is presumably conditioned by the disparity between glycogen breakdown and glycogen formation out of fat

There is accordingly good reason for regarding the non-protein R Q as being chiefly an index of the direction of the dynamic transformation carbohydrate $\rightleftharpoons$ fat   When the reaction is predominantly to the right the R Q is high and when to the left it is low   It is extremely doubtful if the organism ever oxidises muscle glycogen without some compensatory glyconeogenesis and similarly it is also doubtful if, in anabolism, the liver stores glycogen without some transformation of glycogen into fat   One of the modern fundamental deductions in chemistry is that all reactions are reversible and that all reactions tend to reach an equilibrium.   This is also probably true of the phenomena of metabolism   When food-stuffs are combusted *in vitro* the proportion of these is reflected in the R Q but the R Q cannot have this unequivocal significance in the intact organism since concomitant syntheses and decompositions are going on all the time   What fraction of the R Q represents a combustion quotient must for the present remain undecided   Should future investigation determine that the energy requirements of the organism can come only from the direct breakdown of carbohydrate and that fat supplies energy by oxidation through a carbohydrate stage, the non-protein R Q will signify merely, as already stated, the direction of the shift in the dynamic transformation carbohydrate $\rightleftharpoons$ fat, plus the oxidation of the carbohydrate

Conclusions

(1) The administration of 50 grm of glucose causes a leisurely rise in the R Q whereas the administration of equivalent quantities of

sucrose, galactose, lævulose and dihydroxyacetone brings about a prompt rise in the R Q to over unity

(2) These differences cannot be attributed to differences in the rate of absorption as is shown by blood sugar estimations

(3) It is pointed out that these R Q 's cannot be interpreted as indicating a preferential combustion of sugars other than glucose, since the tissues cannot use dihydroxyacetone until this substance has been converted into glucose by the liver

(4) The current conception of the R Q solely, or even mainly, as a combustion quotient is criticised

(5) The suggestion is made that the non-protein R Q represents not a combustion quotient but the algebraic sum of the transformation carbohydrate $\rightleftharpoons$ fat, plus the oxidation of carbohydrate for energy purposes

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FURTHER OBSERVATIONS ON OXYGEN ACCLIMATISATION

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MOST previous observations on animals under altered O_2-pressures in the air have been of comparatively short duration. In a recent paper[1] some observations dealing with prolonged exposures of rabbits to increased and to decreased O_2-pressure in the inspired air were considered In the present paper, this research has been extended, employing monkeys, cats, cavies, mice and rats in addition to rabbits

Technique Most of the details of technique will be found in the paper[1] mentioned above, here we refer briefly to certain modifications Nine experiments (see Table I) were performed, employing three different chambers One of them, called "ordinary small" in Table I, was the same as that used in the previous research and had a capacity of about 169 litres, another termed "ordinary large" was on the same principle, but about four times as large In both these chambers all the experiments (Nos 1 to 8) were carried out under normal barometric pressure In the remaining experiment, No 9, a cylindrical decompression chamber about 6 ft in height and 4 ft in diameter was used at the premises of Messrs Siebe Gorman, Marine Engineers, London, in this experiment the barometric pressure was lowered by gradual stages to about 260 mm Hg, equivalent to an altitude of 30,000 ft , and a group of 30 animals—6 rabbits, 6 rats, 6 cavies and 12 mice—were exposed day and night to these changes for a total period of 33 days The animals were enclosed in their usual cages which were placed in the decompression chamber For purposes of feeding the animals and cleaning the cages, this chamber had to be opened about every 4 days, but the necessary manipulations were carried out rapidly so that the animals were exposed to ordinary air for a total period of about 300 minutes only of the whole 33 days, during the last 7 days under barometric pressure 260 mm Hg the chamber was not opened at all because the animals did not require fresh food, as they had lost their appetites and had not eaten the food last supplied to them The same principle for

feeding and cleaning was followed in all experiments with rabbits, rats, cavies and mice   In the case of cats and monkeys, the chambers had to be opened more frequently, that is, every 2 days instead of every 4 days, by having two sets of trays, cages etc , these manipulations required only a short time, in fact a couple of minutes, if samples of blood etc had to be taken from the animals, an extra 5 or 10 minutes' time was expended

TABLE I. Outline of Experiments.

Exp ref. No	Chamber used	Animals employed	Baro meter mm. Hg	Av O₂ pressure in inspired air mm. Hg	p c.	Approx. altitude corre sponding to O pressure ft	Dura tion days
1	Ordinary small	2 cavies, Nos I and II	745	141	20 1	Sea level	8
			745	330	47 1	—	24
			745	433	61 9	—	33
			745	141	20 1	Sea level	14
2	Ordinary small	1 cat, No II	745	140	20 0	Sea level	7
			745	287	41 0	—	16
3	Ordinary large	3 cats, Nos I, II and III	745	326	46 6	—	11
		3 cats, Nos I, III and IV	745	376	53 7	—	7
			745	140	20 0	Sea level	24
4	Ordinary small	6 rats and 6 mice	745	140	20 0	Sea level	10
			745	297	42·4	—	14
			745	424	60 6	—	21
5	Ordinary large	2 monkeys Nos I and II	745	138	19 7	Sea level	6
			745	311	44 4	—	18
			745	371	53 0	—	11
			745	140	20·0	Sea level	14
6	Ordinary small	2 cavies Nos I and II	745	82	11 7	15 000	10
			745	72	10 3	20 000	7
			745	67	9 5	22 500	7
7	Ordinary large	3 cats, Nos I, III and IV	745	141	20 1	Sea level	6
			745	86	12 3	15 000	14
			745	64	9 1	25,000	15
8	Ordinary large	1 monkey, No II	745	137	19 6	Sea level	4
			745	95	13 6	13 000	12
			745	76	10 9	18 000	7
			745	70	10 0	20 000	6
			745	147	21 0	Sea level	20
9	Decom pression	6 rabbits, 6 rats, 6 cavies, 12 mice	600	116	16 6	7 000	3
			440	85	12 1	15 000	7
			400	77	11 0	18 000	1
			375	72	10 3	20 000	6
			320	62	8 8	25 000	7
			290	56	8 0	27,500	1
			260	50	7 1	30 000	8

The temperature inside the chambers was kept about 15–20° C They were well lighted with electric light or daylight by means of large windows of glass and the animals could thus be kept under observation

In all cases the animals were enclosed in the cages to which they had been accustomed in the laboratory As they had lived for considerable periods in these cages before the experiments commenced, the experiments did not involve any sudden diminution of space for movement The monkeys (*Macacus rhesus*) about 2 kilos in weight had plenty of room to climb about in their cage which measured 67 cm > 61 cm ⟍ 61 cm. In two of the experiments, Nos 3 and 7, three cats were placed in their separate cages in the large chamber, so that they could see one another and had the company of one another, they slept most of the time and when awake, took an interest in what was going on in the laboratory, which they could see through the glass window

For food, monkeys received bananas, apples, oranges, lemons, potatoes, green stuff, tinned milk, biscuits (Spratts), nuts and cod-liver oil, cats received raw and cooked meat, boiled fish and fresh milk, rabbits received hay, oats and cabbage, rats were given oats, barley, bread and milk, and green stuff, mice received much the same as the rats, cavies had bran, grain, hay and greenstuff A very liberal supply of water was placed in all the cages and chambers to prevent dryness, the bedding was kept clean by use of a large amount of sawdust as in the previous research(1)

In five of the experiments, No 1 to 5, the animals were exposed to increased O_2-pressure in the air, the maximum amounting to about 200 p c above normal, in the other four experiments the animals—in some cases the same individuals as used for high O_2-experiments—were exposed to lowered O_2-pressure in the air, the minimum being about 60 p c below normal The general outline of the experiments is given in Table I, to take an example, in Exp No 1 two cavies were exposed in the ordinary small chamber at normal barometric pressure, to 140 mm. Hg of O_2 in the inspired air—that is about normal O_2-pressure —for 8 days, then for the next 24 days they were exposed to increased pressure of O_2 at a level averaging about 330 mm. Hg, this was immediately followed by 33 days exposure to O_2 at 433 mm. Hg, whilst for the last 14 days in the chamber the animals were again under almost normal O_2-pressure for the purpose of control results Exp No 3 followed immediately after Exp No 2 without any break, the change involving increase in the number of cats used and also increase in the size of the chamber employed.

Frequent analyses of the air in the decompression chamber and daily analyses of the air in the ordinary chambers were made, the same precautions being observed as in the previous research[1], O_2-consumption, blood changes and tissue CO_2- and O_2-tensions were estimated by the same methods described in the paper referred to   Blood samples were taken from an ear vein in all animals except rats and mice when a tail vein was used

Effects of altered O_2-pressure in the air upon blood   The increase of Hb p c and of red cells which normally occurs on exposure to lowered O_2-pressure in the air was observed in all my animals except cats Nos III and IV (see Table II and Fig 1)   We are concerned here chiefly with the new results with the opposite condition namely increase of O_2-pressure, I found previously[1] in rabbits a marked decrease of Hb p c and of red cells under increased O_2-pressure and B o r n s t e i n[2] obtained somewhat similar results in a monkey and some dogs in the Elbe tunnel under increased barometric pressure ($+2$ atmospheres)   The present experiments (see Table III and Fig 1) established the general truth of the statement that Hb p c and red cells are decreased
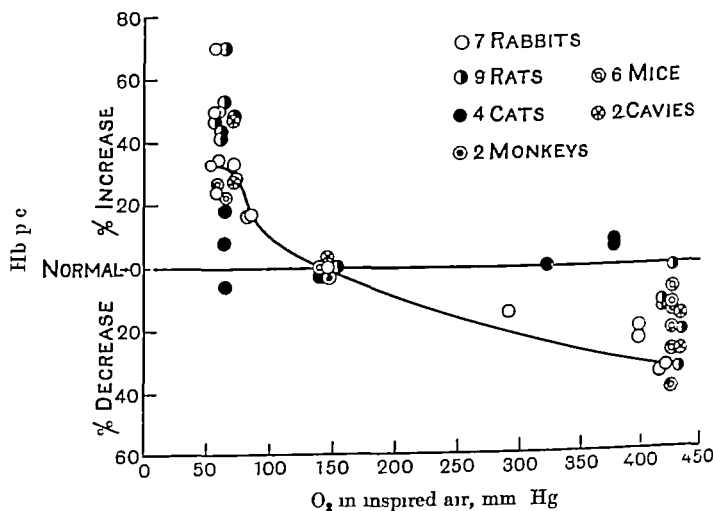

Fig 1   Relations between Hb p c and O_2 pressure in the inspired air during prolonged exposures. The curve is drawn through points taken from one and the same animal, Rabbit No 2 (1)

by increased O_2-pressure in the air, it will be seen that this was so for rats mice, monkeys, and cavies as well as rabbits, cats were the only

TABLE II   Average figures for blood changes under decreased O₂ pressure

Exp ref No	Animals	Red cells mills p.c. mm Normal	Under low O₂	Hb p.c. Normal	Under low O₂	Colour index Normal	Under low O₂	Reticulated red cells, per thousand red cells Normal	Under low O	White cells, thousands p.c. mm Normal	Under low O
9	5 rabbits	4 9	9 3	76	110	1·00	0·76	25	57	9 6	9·0
	6 rats	7 8	13 2	92	138	1·00	0 88	26	62	14 6	17 4
	2 mice	9 2	13 7	96	120	1·00	0·84	12	65	10·5	13 0
8	1 monkey, No II	5 5	8·0	75	100	1·00	0 91	15	35	14 0	12 2
6	Cavy No I	7 5	9·0	100	127	1·00	1·06	20	60	9·0	12 5
	„ No II	6·0	8 5	90	132	1·00	1 03	22	50	12·0	12·0
*	1 rabbit, No II	5 5	9 5	90	120	1 00	0·77	—	—	7 2	10·2
	Averages	6 6	10·1	88	121	1·00	0 89	20	55	10·9	12 3
7	Cat No I	8 7	11 5	85	100	1·00	0 88	—	—	10·7	18·0
	„ No III	7 5	8·0	80	87	1·00	1 02	—	—	16·0	15·0
	„ No IV	7 5	8·0	85	80	1·00	0·89	—	—	14·0	18 0
	Averages	7 9	9 2	83	89	1 00	0·92	—	—	13 6	17 0

* Previous result, see reference (1)

TABLE III   Average figures for blood changes under increased O₂ pressure

Exp ref No	Animals	Red cells mills p.c. mm. Normal	Under high O	Hb p.c. Normal	Under high O₂	Colour index Normal	Under high O₂	Reticulated red cells, per thousand red cells Normal	Under high O₂	White cells, thousands p.c. mm. Normal	Under high O₂
1	Cavy No I	7 5	4 2	100	72	1·00	1 28	20	10	9·0	12 0
	„ No II	6·0	4 5	90	75	1·00	1 11	22	10	12 0	12 0
5	Monkey No I	5 9	4 4	72	58	1·00	1·07	10	5	10·9	10·3
	„ No II	5 5	3 8	75	57	1 00	1 10	15	5	12 2	14·0
4	6 rats	8 2	6·7	97	82	1·00	1·03	23	17	17 3	15 6
	6 mice	9 7	7 1	94	74	1·00	1·07	27	29	12 2	17 7
*	2 rabbits	5 7	3 0	87	57	1·00	1 25	—	—	8 8	10·0
	Averages	6·9	4 8	88	68	1·00	1 13	19	11	11 7	13 1
2, 3	Cat No I	8 7	8 5	85	90	1·00	1·09	—	—	10·7	12 5
	„ No II	7·0	7 5	82	85	1·00	0·96	—	—	9 5	20·0
	„ No III	7 5	9·0	80	87	1 00	0·90	—	—	16·0	25 0
	Averages	7 7	8 3	82	87	1·00	0·98	—	—	12 1	19 2

* Previous results, see reference (1)

animals tested which failed completely to follow the general rule although there were one or two exceptions also amongst the rats and mice (see Fig 1)

Some of the rats and rabbits showed striking increases (70 p c and more) of Hb and of red cells under low O₂-pressure whilst some of the

mice, rats and rabbits exhibited marked decreases (40 p c) under high O_2-pressure (see Fig 1), it was possible by alteration of O_2-pressure in the air to produce nearly three-fold variations in the Hb p c and red cells

The changes I observed, both under low and high O_2-pressure, were produced gradually and passed off again gradually on return to normal O_2-pressure, several weeks being required as shown previously for rabbits[1], this seemed to exclude simple changes in concentration of blood or in storage of red cells   Again Barcroft[3] and others[4] have shown that the reticulated red cells are markedly increased by low O_2-pressures which is regarded as evidence of new red cell formation I obtained a similar increase in the reticulated red cells (see Table II) Moreover, under high O_2-pressure I observed the opposite change, namely a decrease in reticulated red cells (see Table III) in all animals tested except mice   It is possible also that there was an increase in rate of destruction of red cells under high O_2-pressure   Muir and Dunn[5] showed that in hæmolytic anæmia where there was a rapid destruction of red cells, there was a marked increase in iron content of the liver, spleen and kidney   Boycott and Douglas[6] showed that this was observed only in the spleen when the rate of destruction of red cells was slower   I carried out a series of tests with five animals (rats and mice) which had been exposed to high O_2-pressure, using a set of normal rats and mice as controls, I found with the Prussian blue reaction and also by microscopic examination—for remnants of Hb pigment —a greater content of pigmented material in the spleens of the experi mental animals than in those of the controls

Some of the changes in colour index were interesting   There was an increase of 25 p c in colour index of some rabbits and a cavy (see Table III) under high O_2-pressure and a decrease of colour index of about 24 p c under low O_2-pressure (see Table II)   Change in colour index indicates of course a change in the Hb-content of each red cell It was unlikely that a change in value of Hb-content from 0 75 to 1 25 was produced merely by change in concentration of blood

As in the previous research[1] I found no connection whatever between changes in Hb p c and changes in body weight

It has been stated above that the increase of Hb p c under low O_2-pressure passed off in a few weeks after return to normal O_2-pressure, so that any increase of Hb produced by residence at a high altitude soon loses, after a few weeks residence at sea-level, any advantage it may have offered

The leucocyte counts did not show any constant changes (see Tables

II and III), under low O_2-pressure cats Nos I and IV and under high O_2-pressure the mice and cats II and III exhibited marked increases in the number of white cells of the blood, there was no evidence of infection and no rise of body temperature to explain these increases

The differential counts for leucocytes under low O_2-pressure (see Table IV) gave an increase in polymorphonuclear leucocytes as was observed before[1], under high O_2-pressure no great change was observed (see Table V) except in cats II and III where there was the polymorphonuclear leucocytosis mentioned above

TABLE IV Average differential counts for leucocytes under decreased O_2 pressure

		Normal				Under low O_2			
Exp ref. No.	Animals	Poly morphs	Small lympho- cytes	Large lympho- cytes	Mono- nuclears	Poly morphs	Small lympho cytes	Large lympho cytes	Mono nuclears
9	5 rabbits	32	32	23	13	57	32	6	5
	6 rats	17	51	23	9	27	39	26	8
	2 mice	18	33	37	12	28	46	19	7
8	1 monkey, No II	39	35	14	12	75	12	9	4
6	2 cavies	22	45	28	5	30	43	15	12
7	3 cats	40	38	17	5	60	23	10	7

TABLE V Average differential counts for leucocytes under increased O_2 pressure

		Normal				Under high O_2			
Exp ref No.	Animals	Poly morphs	Small lympho- cytes	Large lympho cytes	Mono nuclears	Poly morphs	Small lympho- cytes	Large lympho cytes	Mono nuclears
1	2 cavies	26	33	32	9	27	43	24	6
5	2 monkeys	36	29	24	11	27	51	16	6
4	6 rats	25	62	10	3	23	57	10	10
	6 mice	23	59	12	6	18	62	15	5
*	2 rabbits	23	66	5	6	15	69	6	10
2 3	3 cats	60	27	8	5	81	7	4	8

* Previous results see reference (1)

Effects of altered O_2-pressure upon tissue O_2- and CO_2-tensions The tissue O_2- and CO_2-tensions were estimated as before[1] by injection of N_2 under the skin and into the abdominal cavity and estimating the CO_2- and O_2-tensions in this gas after constancy was established. Cats and monkeys were employed to compare with the previous experiments[1] with rabbits The monkeys were rather small and only small quantities, 100 c c, of N_2 were injected Under high O_2-pressure as

noted previously the gas was absorbed very rapidly so that frequent injections were made   It has been found that if an animal be injected with N_2 in the evening, samples may be withdrawn the next day for experiments provided that previously the animals had several small daily injections for about a week to enable the tissues to become accustomed to the presence of the gas

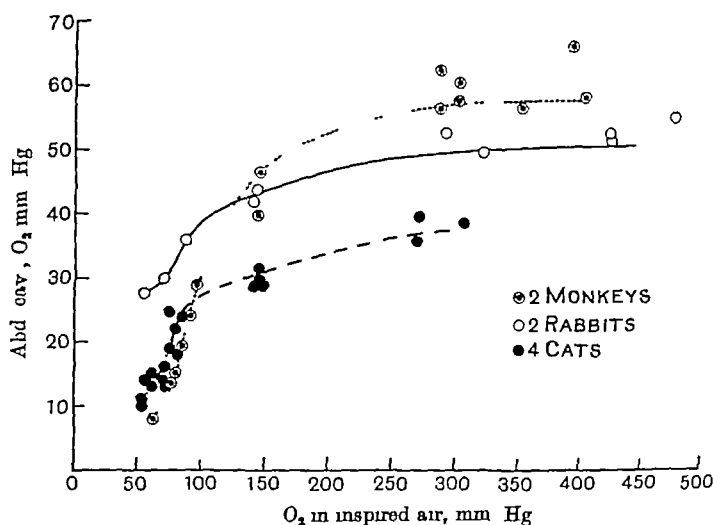In Figs 2 and 3 the results for O_2-tensions have been plotted to

Fig 2   Relations between O_2 tensions in the abdominal cavity and O_2 pressure in the inspired air during prolonged exposures.
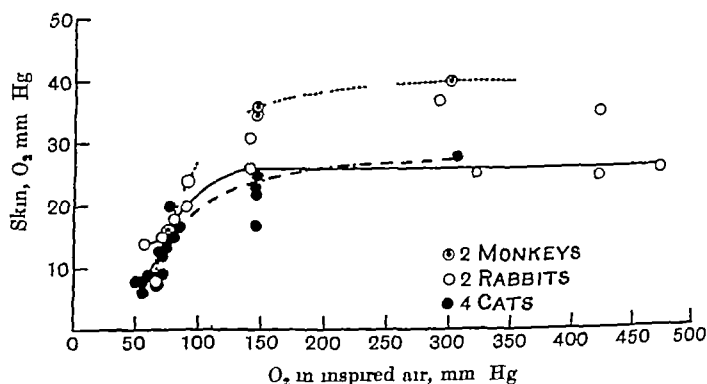

Fig 3   Relations between O_2 tensions under the skin and O_2 pressure in the inspired air during prolonged exposures.

show their relationships with O_2-pressure in the air   Fuller details are given in Tables VI and VII

TABLE VI.   Tissue gas tensions under decreased O_2 pressure

Exp. ref. No	Animal	No of days since beginning of exposure to low O_2	O_2 pressure in inspired air mm. Hg	CO_2 tensions in tissues mm. Hg		O_2 tensions in tissues mm. Hg	
				Skin	Abd. cav	Skin	Abd. cav
7	Cat, No I	—	146	46	46	23	32
		14	84	37	36	17	24
		6	78	34	31	15	22
		2	75	35	32	15	19
		27	68	31	29	9	16
		22	60	27	25	9	15
		29	56	28	27	6	10
		17	54	23	23	8	12
7	Cat No III	—	146	42	43	25	30
		7	75	27	26	20	25
		23	68	28	28	13	14
		20	52	22	22	8	13
7	Cat, No IV	—	146	46	42	17	29
		8	76	31	28	13	19
		24	68	29	29	13	14
		16	62	27	27	8	13
8	Monkey, No II	—	140	39	42	37	40
		7	97	—	38	—	29
		9	91	38	40	24	24
		23	84	—	37	—	19
		5	78	32	37	15	15
		18	74	—	35	—	15
		14	73	—	33	—	14
		16	63	30	33	8	8

Table VII   Tissue gas tensions under increased O_2 pressure

Exp ref No	Animal	No of days since beginning of exposure to high O_2	O_2 pressure in inspired air mm. Hg	CO_2 tensions in tissues mm. Hg		O_2 tensions in tissues mm. Hg	
				Skin	Abd. cav	Skin	Abd. cav
2	Cat, No II	—	146	46	46	23	29
		2	274	46	48	21	36
		4	306	46	48	28	39
		8	269	42	41	35	40
5	Monkey No I	—	145	47	43	35	47
		18	300	—	43	—	63
		25	400	—	46	—	67
		29	300	—	47	—	61
5	Monkey No II	—	145	50	39	37	38
		4	301	48	—	40	—
		17	300	—	46	—	59
		25	400	—	55	—	59
		26	348	—	56	—	57

The chief results with rabbits Nos II and III of the previous research are shown in Figs 2 and 3 for purposes of comparison It will be observed that with all animals the O_2-tensions in the tissues increased as the O_2-pressure in the air increased, the curves for the different types of animals showed on the whole a resemblance in shape but those for the abdominal cavity (Fig 2) were rather more separated from one another than those for the skin (Fig 3) Monkeys and rabbits had normally—that is when breathing O_2 at about 140–150 mm Hg—higher O_2-tensions in the tissues than cats as was shown previously [7]

The shape of the curves depended in the main on the O_2-dissociation curve for blood The points for the rabbit at very low O_2-pressures exhibited a striking difference from those for the cats and the monkey, the former occupying a much higher level than the latter Thus under O_2-pressure in the air about 50–60 mm Hg, the O_2-tensions in the abdominal cavity of the rabbit were about 28–30 mm Hg (Fig 2), whilst those for the monkey and the cats were about 8–10 mm Hg, the O_2-tensions under the skin (Fig 3) showed similar but less marked differences The cats and the monkey were much weakened and very drowsy, whilst the rabbit seemed very much better, the heart was obviously much less affected in the rabbit than in the other animals and circulation was maintained better

Another feature of importance was that the points followed the curves quite independently of the duration of exposure to altered O_2-pressure (Tables VI and VII) The O_2-tensions in the tissues were not brought back to normal level by prolonging the exposure, in other words by acclimatisation, if this had been so the relationships, at least for rabbit No II which acclimatised well (Figs 2 and 3), should be repre sented by almost straight lines and not by curves My experiments proved that acclimatisation to low O_2-pressure was not due to improvement in O_2-tensions in the tissues but to the fact that the tissues became accustomed to the low O_2-tensions surrounding them Rapid acclimatisation is due evidently to the fact that the vital organs, particularly the heart, can tolerate low O_2-tension Similarly under high O_2-pressure in the air, the O_2-tensions (Fig 2) were not reduced to normal by prolonging the exposure and acclimatisation to high O_2-pressure is due to the tissues becoming accustomed to the high O_2-tensions in their immediate environment

The CO_2-tensions in the tissues on the whole increased as the O_2-pressure in the air increased (Fig 4, Tables VI and VII) The points, for both CO_2-tensions under the skin and in the abdominal cavity, for

all animals were fairly close together   The CO_2-tensions in the case of
the monkey did not fall to such a striking degree under low O_2-pressure

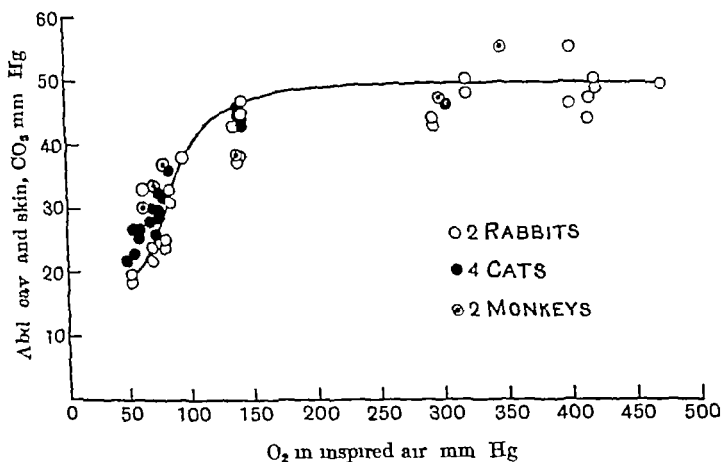Fig 4. Relations between CO_ tensions in the tissues and O_2 pressure in the inspired air
during prolonged exposures   The curve is drawn through points taken from Rabbit
No 2 (1)

as did those for cats and rabbits, this agrees with the fact that in some
men low O_2-pressure does not depress the alveolar CO_2-tension to the
same extent as in others(8)

Since the values for CO_2-tensions in the tissues were much the same
in the cats and rabbits, CO_2-tensions—and therefore breathing—did not
play the chief part in acclimatisation because the rabbits acclimatised
well and the cats did not acclimatise at all

Acclimatisation to low O_2-pressure   No great difference was observed
in the behaviour of the animals under low O_2-pressure produced by
decompression and low O_2-pressure produced by presence of a large
quantity of N_2 Rarefied air did not possess any advantage as is some-
times suggested

Perhaps the simplest indication of acclimatisation is the possession
of normal appetite, capable of maintaining normal weight and normal
O_2-consumption   Table VIII shows the changes in weights and meta-
bolism   There was a distinct loss of weight in all experiments under
low O_2-pressure, indicating that the animals were not fully acclimatised
to the lowest pressures of O_2 tested   The loss of weight was under-
estimated in some cases because the animals were young and growing,
young animals were used since they are stated to acclimatise better

than older animals　The O_2-consumption was not quite so good a guide as the weight, it was estimated over practically the whole period of the experiment but was not for basal conditions since it included effects of feeding and movement　The figures were most useful and important

TABLE VIII. Average figures for metabolism and weight under decreased O pressure

Exp ref No	Animals	Duration of exposure to low O$_2$ days	O consumed c c per min. per animal			Weight per animal gm.		
			Normal	Under low O	Alteration p c	Normal	Under low O$_2$	Alteration p c
9	5 rabbits	33	—	—	—	2486	1864	−25 0
	6 rats	33	—	—	—	131	104	−20·6
	2 mice	33	—	—	—	21	16	−23 8
8	1 monkey, No II	25	24 3	18 5	−23 9	1720	1430	−16 8
6	2 cavies	24	13 7	9 5	−30 6	827	685	−17 1
9	6 cavies	17	—	—	—	304	260	−14 0
*	1 rabbit	43	28 5	27 1	− 4 8	2750	2400	−12 7
7	Cat No I, , No III, „ No IV	29⎫ 22⎬ 28⎭	21 9	14 2	−35 1	⎧2710 ⎨2730 ⎩2200	1670 1480 1120	−38 3 −45 7 −49 1

* Previous results see reference (1)

since they fully confirmed the changes in weight, the animals did not appear to change their habits of feeding and movement to any degree from time to time　Where there was a loss of weight under altered O_2-pressure, the experiment was continued in the chamber under normal O_2-pressure to prove that living in the chamber was not responsible for this loss of weight, under normal O_2-pressure the fall of weight soon ceased and there was a gradual return to normal appetite and weight whilst still in the chamber　There was no evidence that animals accus tomed to laboratory life were affected adversely by mere enclosure in the chambers used and for the durations tested

Although it was proved that none of the animals became acclimatised to O_2-pressure about 50 mm　Hg, that is equivalent to the O_2-pressure at the top of Mount Everest, nevertheless 5 rabbits, 6 rats and 2 mice, after a certain degree of training, survived 7 days' continuous exposure to 50 mm　Hg pressure of O_2 (see Exp　9, Table I)　It was obvious then that some mammals can exist for at least a week under O_2-pressure similar to that on Mount Everest, the animals had lost their appetites and were deteriorating rapidly although the rats and mice exhibited activity at infrequent intervals, climbing about their cages　The rate of breathing was increased slightly, in some cases about 30 p c, but

there was no obvious respiratory distress   The survivors certainly looked more vigorous and capable of movement than did similar animals which were exposed suddenly to low O_2-pressure at about 50 mm Hg for 6 hours, without any previous training   In this sudden decompression, the animals were exceedingly weak and drowsy exhibiting marked hyperpnœa, yet all except one survived the 6 hours test, 2 cats, 2 finches, 5 rabbits, 6 rats, 6 cavies, and 12 mice constituted the survivors, 1 rabbit dying

It was observed in the prolonged experiments that some rabbits, rats and mice maintained their appetites and weights at 70 mm Hg pressure of O_2 (20,000 ft) and seemed to have become acclimatised to that pressure, but the cats and the monkey lost their appetites completely before this level and could not tolerate such a low pressure of O_2, losing weight rapidly, cavies also fared badly   Dr J S Haldane informed me that cats cannot live even on Pike's Peak (14,000 ft)

We have already seen (Figs 2 and 3) that the O_2-tensions in the tissues of the monkey and the cats were much lower than those of the rabbit No II, during the exposure to very low O_2-pressure in the air and it is considered that heart failure in the monkey and cats was responsible   The differences between the animals existed from the first days of exposure and were not at all dependent on the length of time of exposure   These differences were not connected in any way with the powers to increase Hb, thus rabbit No II(1) and monkey No II exhibited similar increases (about 33 p c) in Hb p c after several weeks' exposure to low O_2-pressure yet rabbit No II tolerated the change better than any other animal tested whilst monkey No II was the least able to withstand it, exhibiting great weakness

As was proved before with rabbits(1) increase of Hb p c , when it did occur, produced no improvement in tissue O_2-tension under low O_2-pressure in the air   Thus cat No I showed an increase of nearly 20 p c in Hb p c and of 32 p c in red cells whilst cats Nos III and IV showed much smaller changes, yet in all three cats the changes in tissue O_2-tensions were much the same under very low O_2-pressure (see Table VI) What then was the value of the increase in Hb p c ? Obviously it allowed the blood to carry more O_2 per c c and this in itself would relieve the vital organs, heart, etc , in their efforts to supply the necessary O_2, cat No I was definitely more resistant than cats Nos III and IV   It was obvious that the absolute value of O_2-tension in the tissues did not decide the issue since all three cats had the same O_2-tensions in the tissues (see Table VI) It is suggested that the deciding factor was the

ability of the heart of cat No I to continue to function under the low O_2-pressure, undoubtedly in this case the extra Hb was of value

Haldane(9) pointed out some years ago that acclimatisation often occurs rapidly and long before there is any change either in Hb or in certain other factors often credited with much importance Somervell(10) found that some native porters with low values of Hb p c were more efficient at high altitudes than European members of the expedition with much higher Hb p c Again if we take loss of weight as an indicator of the powers of acclimatisation to low O_2-pressure we shall see that changes in Hb p c had no connection with acclimatisation, thus in six rabbits the losses of weight in percentages were 13, 14, 19, 30, 30 and 32 respectively whilst the percentage increases in Hb p c were 33, 22, 50, 50, 34 and 71 respectively All the evidence thus suggests that increase of Hb p c is merely a consequence or symptom connected with prolonged exposure to low O_2-pressure and although of value was not the factor invariably controlling acclimatisation.

Barcroft(11) has reviewed the evidence regarding the adverse effects of low O_2-pressure upon the efficiency of the heart That the heart was the chief organ concerned in the power of the body to tolerate very low O_2-pressure in my experiments was evident from post-mortem examination of the organs of 2 cats, 1 rabbit, 7 cavies and 5 mice Nerve cells were but little changed from normal The heart in all cases showed definite evidence of failure, being dilated, flabby and exhibiting in varying degree a degeneration similar to the fatty change described for cavies by Rosin(12) Degenerative and necrotic changes in the liver and kidney indicated lessened circulation due to heart failure, as did the general state of marked congestion of the organs Why the heart in some animals was more efficient under low O_2-pressure was not obvious, none of the present theories of acclimatisation solved the problem, some inherent quality of the muscle fibre must be searched for

An interesting change was observed perhaps indicating a physiological adaptation, the tunica muscularis of the branches of the pulmonary artery were markedly hypertrophied (see Fig 5) in the cats, also the plain muscle fibre in the interalveolar septa was greatly increased The condition was not observed in normal cats but it was noted in a cat suffering from O_2-deficiency due to broncho-pneumonia It is possible that the change represented an adaptation to increased pressure in the pulmonary circulation due to inefficiency of the left ventricle, or perhaps the increase of muscular tissue aided the onward flow of blood through the lungs by rhythmical contraction Whatever

the explanation it was evident that tissue reproduction could occur at very low O_2-tensions

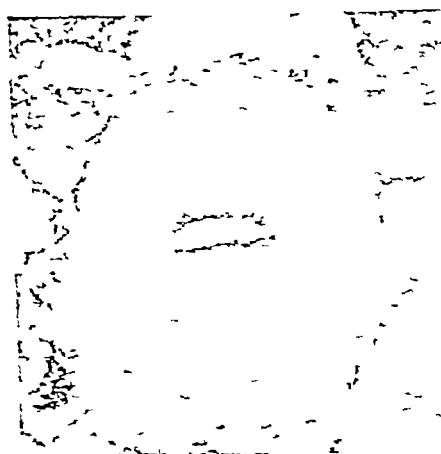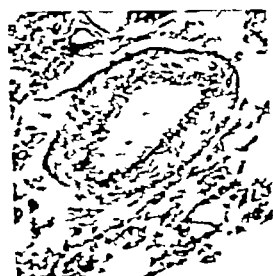<div align="center">Fig 5 A.   Fig 5 B</div>

Fig 5 (A) Transverse section of branch of pulmonary artery of cat No III showing hypertrophy of tunica muscularis after exposure to low O_2 pressure. H. and E × 125

(B) Transverse section of similar artery from a normal cat H. and E. × 125

Acclimatisation to increased O_2-pressure Lorraine Smith[13] and others[14] have proved that animals die from a type of pneumonia if suddenly exposed for any length of time to O_2-pressure at or above 490 mm. Hg (70 p c of an atmosphere) I[1] found that rabbits survived very prolonged exposures to somewhat lower pressures, namely 420 mm Hg (60 p c of an atmosphere) About the same time Barach[15] obtained similar results with rabbits In the present experiments it was proved that monkeys, cavies, rats and mice also tolerated O_2-pressure at 420 mm Hg for prolonged periods without great loss of weight (Table IX) Monkey No I, one of the rats and five of the mice gained in weight, but one of the rats and one of the cavies lost considerable weight Most of the above animals showed no great loss of appetite but a striking difference was observed with cats, they did not appear to tolerate O_2-pressure at 300 mm. Hg (about 40 p c of an atmosphere), losing their appetites and becoming exceedingly sleepy, the loss of weight was great (see Table IX) but apart from general weakness and drowsiness there was no obvious symptom, body temperature was normal until the O_2-consumption had fallen greatly, then the body temperature also fell

TABLE IX. Average figures for metabolism and weight under increased O_2 pressure.

Exp ref. No	Animals	Duration of exposure to high O_2 days	O_2 consumed c c. per min. per animal			Weight per animal gm		
			Normal	Under high O_2	Alteration p c.	Normal	Under high O_2	Alteration p c
1	Cavy No I	59	13 5	10 2	−24 4	850	670	−21 1
	,, No II	59				670	630	− 6 0
5	Monkey No I	29	27 7	23 0	−16 9	2210	2220	+ 0·4
	,, No II	29				1985	1890	− 5·0
4	6 rats	35	35 9*	36 6*	+ 1 9	193	179	− 7 2
	6 mice	35				26 8	30 7	+14 5
†	2 rabbits	31	28 7	26 5	− 7 3	2875	2625	− 8 7
2, 3	Cat No I	18	29 7	18 7	−37 0	3050	2300	−24 6
	,, No II	27				3100	1570	−49 3
	,, No III	18				3220	1570	−51 2

* Total c.c. per min for 6 rats and 6 mice
† Previous results, see reference (1)

definitely, there was no infection in the blood and no obvious injury to the organs except the lungs which exhibited some collapse and congestion and the presence of a few catarrhal cells Although there was no definite evidence it might be conceived that some poison was formed by the action of the high O_2-pressure upon the lung epithelium and general poisoning resulted, the cats for some reason could not antagonise this "poison" so well as rabbits, rats, mice, monkeys and cavies Perhaps the diet was concerned The absolute values of the O_2-tensions in the tissues (Figs 2 and 3) under increased O_2-pressure in the air did not control the powers of toleration, since the tissue tensions were much higher in the monkeys and rabbits than in the cats, and yet the cats were so much more sensitive to the change

The changes in Hb p c were no accurate guide to the powers of acclimatisation to high O_2-pressure as judged by general appearance, change in weight, etc Thus one of the mice showed scarcely any decrease in Hb p c yet its increase (25 p c) in weight was greater than that of any other mouse or any other animal tested Changes in Hb p c under increased O_2-pressure were possible consequences and not the essential factor controlling acclimatisation Acclimatisation to high O_2-pressure in the air is due to the tissues becoming accustomed to the effects of the abnormally high O_2-tension in their immediate environment, even when the Hb was greatly decreased in the rabbits and the monkeys the O_2-tensions were still abnormally high (see Tables III and VII and also previous results(1))

SUMMARY

1 Some mammals—rabbits, rats and mice—after a certain degree of acclimatisation can exist in a decompression chamber for at least 7 days under continuous exposure to low O_2-pressure equivalent to that at the top of Mount Everest, the animals exhibited some activity but were deteriorating rapidly Cats, a monkey and cavies tested could not tolerate such a low O_2-pressure for such a time

2 Unlike most animals cats could not tolerate prolonged exposure to high O_2-pressure at 420 mm Hg (about 60 p c of an atmosphere) They lost appetite and deteriorated rapidly, general weakness being the only obvious change and a general poisoning being suspected, it is suggested that the poison is formed in the lungs although the lungs showed only some collapse and congestion

3 Decrease of Hb p c and of red cells occurs as a rule under exposure to increased O_2-pressure in the air with normal barometric pressure, in monkeys, rabbits, rats, cavies and mice, but was not observed in any of the cats tested The general rule that Hb p c decreases as the O_2-pressure in the air increases and *vice versa* is thus established It is possible to produce nearly three-fold variations in the Hb p c and red cells by alteration in O_2-pressure in the air Any change produced by altered O_2-pressure in the air passes off again, after a few weeks' exposure to normal O_2-pressure

4 Changes in Hb p c are not essential to acclimatisation to changes in O_2-pressure in the air and should be regarded only as possible consequences, they are of value to the heart when they do occur, at least under low O_2-pressure

5 Contrary to prevailing views acclimatisation to lowered O_2-pressure in the air is not due to improvement in tissue O_2-tension, what really occurs is that the tissues become accustomed to the low O_2-tension in their immediate environment Rapid acclimatisation is due to the ability of the vital organs particularly the heart, to continue to function under a low O_2-tension, in all animals affected by low O_2-pressure heart failure was the most constant phenomenon.

6 Acclimatisation to increased O_2-pressure in the air is due chiefly to the tissues becoming accustomed to the effects of an abnormally high O_2-tension in their immediate environment, tissue O_2-tension is not reduced to normal.

7 An hypertrophy of the tunica muscularis of the branches of the pulmonary artery was observed under exposure to low O_2-pressure

I am indebted to Dr Leonard Hill and to R H Davis, Esq, Managing Director of Messrs Siebe Gorman, London, for facilities in the performance of the above experiments   Thanks are also due to Capt S R Douglas and Dr P P Laidlaw for advice *re* the pathological changes, and to Messrs F Thatcher and G Probent, the Engineers who attended day and night to the decompression chamber at Messrs Siebe Gorman's

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THE HYDROGEN-ION CONCENTRATION OF BLOOD CORPUSCLES By HAROLD TAYLOR

(From the Physiological Laboratory, Cambridge)

SOME work has been done previously (Taylor[25]) on the possibility of using the Donnan membrane equilibrium as a means of investigating the ions inside the blood corpuscles The determinations in those cases were done at room temperature and very few precautions taken against loss of CO_2, and on the whole the results were of more qualitative than quantitative importance It was also desirable that the quantity of blood used should be not more than 30 c c so that one subject could supply the blood for the whole determinations The determination of the hydrogen-ion concentrations by means of an electrometric method would then give a true electrometric measure of the hydrogen-ion concentration inside the corpuscle

PREVIOUS DETERMINATIONS

The preliminary work of Hasselbalch and Lundsgaard[13], Konikoff[17], and Milroy[21] all points to the corpuscles being more acid than the serum Barcroft, Bock, Hill, Parsons, Parsons and Shoji[1] conclude from the oxygen dissociation curves of different bloods that the pH of the corpuscles is probably different from the pH of the serum

Calculations of the pH of the corpuscles using the equations of Henderson[14] and Hasselbalch[12] have been made by Campbell and Poulton[2] from the data given by Joffe and Poulton[16], Friedericia[10], Warburg[30], and Van Slyke, Wu and McLean[26] Van Slyke, Hastings, Murray and Sendroy[27] as well as Warburg[30] have made electrometric measurements on hæmolysed corpuscles Conway and Stephens[4] have applied the colorimetric method of Dale and Evans[5] to the case of hæmolysed corpuscles

With the exception of Parsons[23] the whole of the investigators find that either hæmolysed blood or hæmolysed corpuscles are more acid than the serum or whole blood The quantitative values are very doubtful as in no case has a true activity measurement been made on oxygenated corpuscles, as this can only be obtained by a wholly electrometric method.

The calculations from the Hasselbalch-Henderson equation are unreliable, owing to the uncertainty of the value of the constant pK', and the possibility of this factor not being a constant Electrometric measurements, where the hydrogen electrode is placed in hæmolysed corpuscles, are probably erroneous owing to the high protein concentration and the difficulty of removing the last traces of oxygen

THEORY OF METHOD

The theory of the method has been given previously (Taylor[25]) It is in practice an extension of the dialysis-colorimetric method of Dale and Evans[5], using hæmolysed corpuscles and measuring the hydrogen-ion concentration electrometrically

Donnan[7] has shown that if there is an indiffusible ion on one side of a membrane in the presence of diffusible ions, an unequal distribution of diffusible ions will take place on the two sides of the membrane This unequal distribution of diffusible ions will cause an electrical potential difference to be set up between the two sides of the membrane, and the magnitude of the potential difference depends upon the concentrations of the diffusible ions Loeb[18, 19] has applied Donnan's formulæ to the case of protein solutions, and found that they would apply to the hydrogen and chlorine ions A V Hill[15] has pointed out that Donnan's formula will hold for any equilibrium which involves an unequal distribution of ions, and suggests the possibility of the use of the membrane potential as a means of analysing complex solutions

For the hydrogen ions the formula will be

$$E = \frac{RT}{nF} \log_e \frac{[\mathrm{H}]_1}{[\mathrm{H}]_2}$$

Adopting Sorensen's notation we can substitute $p\mathrm{H}$ for $-\log_{10} [\mathrm{H}]$ and we get, introducing numerical values for R, n and F
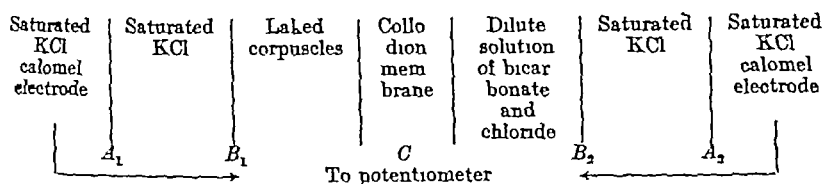
$$E = 0\ 00019837\ T\ [p\mathrm{H}_2 - p\mathrm{H}_1]\ \text{volts}$$

This gives the relationship between the $p\mathrm{H}$'s on both sides of the membrane The sign of the potential difference will depend upon whether the indiffusible ion is an anion or a cation The sign of the potential difference can be shown to be related to the sign of the charge on the protein ion in such a way that the side containing the indiffusible ion is positive when that ion is a cation and negative when it is an anion (Michaelis[20])

This can then be applied to the case of blood corpuscles If the protein solution is hæmolysed blood corpuscles and the potential difference measured between them and a crystalloid solution in "diffusion

equilibrium" with them across a collodion membrane, the hydrogen-ion activity of the corpuscles can be calculated from the hydrogen-ion activity of the crystalloid solution. This will apply equally well for oxygenated as reduced corpuscles As the measurements of the pH and the membrane P D are electrometric, the results obtained will represent true activities

The membrane potential is measured by means of two saturated KCl calomel electrodes, one on each side of the membrane, with saturated KCl as the contact fluid The cell employed is as follows

Saturated KCl calomel electrode	Saturated KCl	Laked corpuscles	Collo dion mem brane	Dilute solution of bicar bonate and chloride	Saturated KCl	Saturated KCl calomel electrode
A_1	B_1		C		B_2	A_2

To potentiometer

The only potential difference measure will be that at the membrane C, as with saturated KCl as contact fluid the boundary potentials at B_1 and B_2 are nil, whilst the potential differences at A_1 and A_2 cancel each other as they are both of the same magnitude A_1 and A_2 are rarely equal in practice, but the difference between them can be estimated and the necessary correction made

The above theory assumes that hæmolysis makes no difference to the reaction of the corpuscles If the corpuscles are laked by freezing them and thawing them it is unlikely that the hydrogen-ion concentration will be changed, as this method of laking does not involve the introduction of any other body

THE HYDROGEN-ION ACTIVITY OF HUMAN BLOOD CORPUSCLES

To prevent a large dilution of the corpuscles by the dialysing fluid, it is necessary to have the volume of the hæmolysed corpuscles large compared with the volume of the dialysate To do this it is necessary to place the dialysate in a collodion thimble and suspend this in the hæmolysed corpuscles as by this means the ratio of corpuscles to dialysate can be made large indefinitely This means that the osmotic pressure of the protein will tend to cause the membrane to collapse, but it is found that the membranes made as described later will hold until diffusion equilibrium has been set up If the corpuscles are placed inside a collodion thimble it is found that the least practicable volume of dialysate in which they can be suspended is far too large

In the earlier experiments with sheep blood the volume of the blood used was of the order of 150 c c , which gave approximately 50 c c of hæmolysed corpuscles In these cases the membrane potential measured with liquid contacts was quite stable, the diffusion into the corpuscles and the dialysate (about 9 c c) being negligible In order to carry out the experiments on human blood the amounts of blood and dialysate taken each time have to be reduced to 25 c c and 1 c c respectively It is found that the diffusion of the KCl from the electrodes in this case is sufficient to cause unstable readings This has been remedied by using agar contacts on the calomel electrodes, the agar being dissolved in saturated KCl solution The agar contacts do not alter the measurements of the membrane potential, and show no sign of diffusion of the KCl, as is shown by leaving them in distilled water for two days, when the water gives only a faint cloudiness with silver nitrate solution

In the actual experiment the hydrogen-CO_2 mixture for the hydrogen electrode is made up first and analysed in a Haldane apparatus Enough of the mixture is made to allow a hydrogen electrode measurement to be made on both the serum and the dialysate The mixture is made up approximately to the required concentration and the exact composition determined by analysis

Between 25 c c and 30 c c of blood are taken from the arm by a hypodermic syringe and defibrinated by whipping with a feather The blood is then filtered through glass wool To prevent the formation of lactic acid during the experiment 0 3 c c of a 1 p c sodium fluoride solution are added This amount of fluoride is sufficient to keep the pH of a sample of blood constant for 5 to 6 hours at 37 5°

The blood is equilibrated with CO_2 in two Barcroft tonometers, whose volumes are each slightly more than 300 c c The tonometers are filled at room temperature and the CO_2 run in from a burette The amount of CO_2 added is such that it will give the same CO_2 pressure as present in the hydrogen mixture, when the tonometer is heated to 37 5°

The blood is then equilibrated with the CO_2 by revolving the tonometers for half-an-hour in a water bath at 37 5° The amount of oxygen present is always sufficient to oxygenate fully the hæmoglobin After equilibration the blood is centrifuged under a layer of paraffin The centrifuge is surrounded by a thermostat and is heated for a considerable time before use to bring it to the correct temperature The metal cap and the glass tube containing the paraffin are also heated before the blood is transferred into them As the tonometers have been filled at room temperature there is always an excess pressure in them, which is

sufficient to drive out all the blood on turning the tonometer tap   The blood is transferred from the tonometer to underneath the paraffin without loss of CO_2

After centrifuging for half-an-hour the separation of corpuscles and serum is as complete as possible with a centrifuge which runs at 4000 revolutions per minute   The separation is almost absolute, but the hæmatocrite value is never quite reached   The error due to this small amount of serum remaining is certainly much less than 0 01 pH, which would be the error if the corpuscles were not buffered by the hæmoglobin.

The serum is then drawn off from above the corpuscles and both corpuscles and serum treated as separate phases   The separated corpuscles are laked by freezing them and subsequently thawing them   The freezing is done in a small flask which is revolved in a freezing mixture of ice and salt   The freezing is continued until the corpuscles have the appearance of a dull pale red mass   They are then thawed by immersing the flask in water, the temperature of which is not more than 37 5°   The liquid obtained should be quite clear and if not the whole must be re-frozen

The shaking during the freezing and the thawing causes a loss of CO_2 from the corpuscles which has to be restored.   This is done by re-equilibrating them in a Barcroft tonometer with the CO_2 pressure used originally

The laked corpuscles are then dialysed against a crystalloid solution.   For human blood the solution placed in the collodion tube is $0 9 \times N/10$ KCl and $0 1 \times N/10$ NaHCO$_3$, which has been equilibrated with the required CO_2 pressure to accelerate the equilibrium   The volume of the corpuscles is generally about 7 c c to 8 c c and the collodion membrane contains 1 c c of the crystalloid solution   The collodion membranes are made on the outside of a small tube, with a hole blown in the end, the method being similar to that used by Sörensen[24]   The collodion is made in a 5 p c solution, the solvent being 75 p c alcohol and 25 p c ether (by volume)   The membrane is made in three coats and then allowed to dry for 1½ hours before immersing in water

During the dialysis the corpuscles and dialysate are placed under a large bell-jar in which the necessary CO_2 pressure has been set up, and the whole is placed in an air bath at 37 5°   The electrodes, membrane with crystalloid solution and tube to contain the corpuscles are placed in the bell-jar first and the whole heated to 37 5°, and the necessary

CO_2 pressure set up The corpuscles are then transferred from the tono-meter to the dialysing apparatus through a tube with a tap in the side of the bell-jar The excess pressure in the tonometer is always sufficient to empty the tonometer on opening the tap In this way there is no loss of CO_2 or any cooling during the transference

As the ends of the electrodes are blocked with agar the electrode contacts can be left in the dialysing apparatus throughout the dialysis and the potential difference measured continually without disturbing the bell-jar

The dialysis is allowed to continue until the potential difference across the membrane becomes constant This generally is attained in about $3\frac{1}{2}$ hours, but varies slightly in different experiments The dialysis is continued until the potential difference is constant over half-an-hour, which is taken as indicating that diffusion equilibrium has been attained Any differences in the electrodes are measured by placing both in the same solution

Equilibrium having been set up the bell-jar is quickly removed and the collodion sack containing the dialysate immediately removed from the laked corpuscles The pH of the dialysate is then found by a hydrogen electrode, the hydrogen containing the same CO_2 pressure as the dialysate and the corpuscles The hydrogen electrode is kept in a water bath at 37 5° and the measurements made against a calomel electrode saturated with KCl at 37 5° The hydrogen electrode used is similar to the one described by Parsons(23), and a potentiometer graduated to 0 1 milli-volt is used to measure both the electrode potentials and the membrane potentials

The hydrogen-ion concentration of the corpuscles is calculated from the formula

$$E = 0\ 00019837\ T\ [p\text{H (dialysate)} - p\text{H (corpuscles)}]\ \text{volts},$$

where E = potential difference and T = absolute temperature As all the measurements are electrometric the pH obtained is a true measure of the activity of the hydrogen-ion

The pH of the separated serum is also found by the same electrode, using the same hydrogen-CO_2 mixture

RESULTS

The results obtained for human blood are given in the following table The potential of the hydrogen electrode in each case has been corrected to 760 mm of hydrogen

TABLE I.

No. of Exp	1	2	3	4	5	6	7	8	9	10	11
Pressure of CO_2 (in mm.)	43·5	115	62	47	175	20·5	35	18	60	27	49
Membrane potential dialysate positive (millivolts)	5·0	4·6	3·7	2·9	3·1	3·6	4·8	4·8	1·5	2·0	2·2
pH of serum	7·33	7·70	7·26	7·33	7·55	7·54	7·44	7·59	7·26	7·51	7·29
pH of dialysate	7·22	7·54	7·01	7·11	7·43	7·30	7·27	7·50	7·03	7·23	7·12
pH of corpuscles	7·14	7·47	6·95	7·06	7·38	7·24	7·19	7·42	7·00	7·19	7·08
$\dfrac{cH\ (corpuscles)}{cH\ (serum)}$	1·55	1·70	2·04	1·86	1·48	2·00	1·78	1·48	1·82	2·09	1·62

Human blood

The potential of the hydrogen electrode has been measured against a saturated KCl calomel electrode which has been checked by a buffer solution which is $N/10$ for both sodium acetate and acetic acid. The potential difference between a hydrogen electrode in a solution normal to hydrogen-ions and a standard saturated KCl, calomel cell has been taken as 235 2 millivolts at 37 5° (Michaelis[20])

The graphs (Fig 1) give the variation of the pH of the corpuscles with varying CO_2 pressures, and also the pH of the separated serum
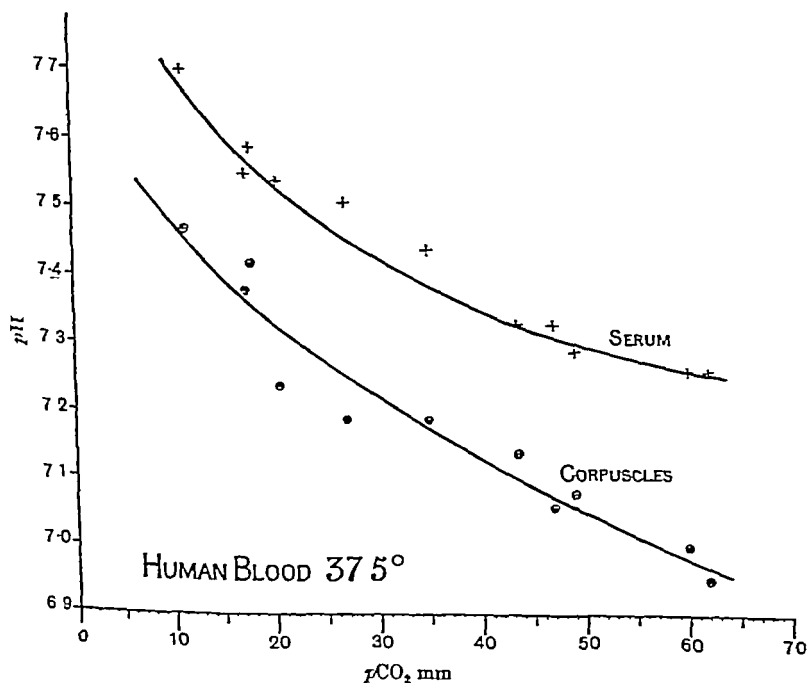

Fig 1

THE HYDROGEN-ION ACTIVITY OF GOOSE CORPUSCLES

The corpuscles of man and all mammals are non-nucleated except when newly formed, and very few nucleated corpuscles are present in the blood On the other hand, the corpuscles of various cold-blooded animals and of birds possess definite nuclei during the whole of their life-time in the blood It is quite possible that this nucleus plays some part in the buffering of the blood and the relationship between the corpuscles and the plasma may be totally different for the non-nucleated corpuscles In this case the curves relating the pH's of the corpuscles and serum with the CO_2 pressure may differ in shape from those of human blood

It was proposed to determine the pH of the corpuscles of a bird's blood over a range of CO_2 pressures and compare the results obtained with the results obtained on human blood There is a possibility that some idea of the functions of the nucleus may be obtained

The bird used for these experiments was a goose, which, being a fairly large bird can be bled from time to time to the extent of 25 c c The blood volume of a goose is large compared to the body weight and the bird itself is easily kept and does not require any special attention to keep it in good condition

The nucleated corpuscles are definitely living cells, and have a very appreciable respiration rate Oxygenated corpuscles lose their oxygen on standing and become more acid due to the corresponding production of CO_2 In the pH determinations this auto-oxidation had to be prevented by means of a small amount of KCN

The blood is extracted from the goose by inserting a hypodermic needle into one of the veins of the wing in the same way as is done for human blood The underside of the wing is comparatively free from feathers and is covered only by a layer of down, which can be plucked off easily The skin can be exposed for a considerable distance and the veins are easily discernible The largest vein is one about midway between the joint in the middle of the wing and the body of the bird, the vein showing up very well as it passes over the bone The vein when punctured is very liable to collapse, and the blood can be obtained only by blocking the vein beyond the point where the needle has been inserted Owing to the arrangement of the bones a tourniquet is not very effective and the best method of blocking the vein is to pinch it by hand To get a clearer view of the veins and also an increased venous pressure the goose is always turned on its back In many cases the

constriction of the veins is so effective that only a few c c of blood can be obtained.

The blood is treated with 0 3 c c of 2 p c sodium fluoride solution and a similar amount of 2 p c potassium cyanide solution, and then defibrinated by whipping The clot tends to break up into small pieces and it is always necessary to strain the blood through glass wool.

The remainder of the treatment is identical with the treatment in the case of human blood except the temperature is $42°$, which is the body temperature of the goose When the blood is centrifuged a better separation of corpuscles and serum is obtained than in the case of human blood

O Warburg[31] has shown that when birds' corpuscles are frozen and thawed at a temperature of not more than $42°$, the nuclei remain intact The presence of the nuclei can be demonstrated easily under the microscope The nucleated corpuscles seem to be reversibly hæmolysed as is the case with mammalian blood It is interesting to note that on the addition of salt solution the corpuscles apparently re-form round the nuclei.

The same crystalloid solution is used in the collodion tube during dialysis as in the case of human blood since the base is mostly potassium The hydrogen-ion concentrations of the serum and the dialysate are found by the hydrogen electrode working at $42°$, the calomel cell being saturated with KCl at $42°$

The pH of the goose corpuscles is then calculated from the formula given previously, which becomes at $42°$

$$E = 0.0625 [pH \text{ (dialysate)} - pH \text{ (corpuscles)}] \text{ volts}$$

RESULTS

The results are given in the following table The hydrogen electrode values have been corrected to 760 mm of hydrogen. The value for the potential difference between a saturated KCl calomel electrode at $42°$

TABLE II.

No of Exp	1	2	3	4	5	6	7	8	9	10	11	12
Pressure of CO₂ (in mm.)	9	35·5	45	56	14 5	30	16	15·0	34 5	-0	27	57
dialysate potential pressure (mm Hg)	3·0	4 2	2·6	2 8	4 2	4·6	4 4	4 5	3 3	2·9	—	—
pH of serum	7 84	7 46	7·40	—	7 60	7 53	7 56	7·65	7 39	7·41	7 51	7 37
pH of dialysate	7·64	7 26	7 15	7 12	7 42	7 33	7 43	7 45	7 22	7 25	—	—
cH (corpuscles)	7 59	7 19	7 11	7·08	7 35	7 26	7 36	7 38	7 17	7 20	—	—
cH (serum)	1 76	1 86	1·95	—	2·04	1 86	1 59	1 86	1 66	1 62	—	—

Goose blood

and a solution normal for hydrogen ions at that temperature has been taken as 233 0 millivolts   Michaelis' values[20] do not go beyond 38° and this value has been obtained by extrapolation   Owing to the accidental death of the first goose, two geese have been used to obtain the curve (Fig 2)
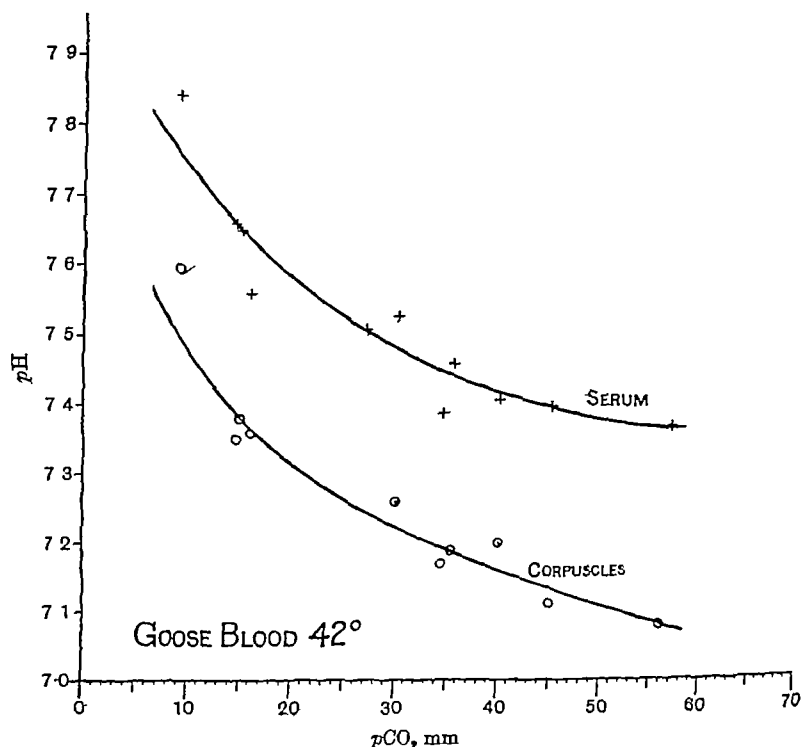

Fig 2

DISCUSSION OF RESULTS

The graphs given show the variation of the pH's of the corpuscles and the serum over the physiological range of CO_2 pressures   The corpuscles are definitely more acid than the serum during the whole of the physiological range   In the case of both human and goose blood the difference of pH between the serum and the corpuscles does not become less as the serum becomes more acid, but if anything increases slightly Campbell and Poulton[2], Friedericia[10] and Van Slyke, Wu and McLean[26] found that the difference in pH between the corpuscles and the serum decreased as the blood became more acid   In all these

cases the Hasselbalch-Henderson equation has been used Van Slyke, Hastings, Murray and Sendroy[26] using electrometric measurements on reduced corpuscles find that the difference decreases slightly as the serum becomes more acid

The equilibrium at the corpuscular membrane is treated in the discussions of Warburg[30] and Van Slyke, Wu and McLean[26] as a kind of Donnan equilibrium and the relationships worked out as in the case of one indiffusible ion It is almost certain however, as shown by the work of Hamburger[11], Doisy and Eaton[6], Ege[5], that the corpuscular membrane is permeable to anions, but not to cations Therefore inside the corpuscle there are indiffusible cations besides the indiffusible protein anion, whilst outside the corpuscles are indiffusible cations Wu[32] points out that the osmotic stability of the corpuscles depends upon the indiffusibility of the cations It is quite logical to work out a Donnan equilibrium arrangement depending upon the indiffusibility of the cations independent of the hæmoglobin Consequently the equilibrium at the corpuscular membrane will be of a complex type depending upon the indiffusible protein anion and the indiffusible cations inside the corpuscle and the indiffusible cations in the plasma, and perhaps the plasma proteins In this case the formulæ for a Donnan equilibrium of one indiffusible ion may not hold Thus it is quite possible that even if the ratio of chloride ions across the corpuscular membrane approaches unity with increasing acidity, the hydrogen-ions will not tend to equalise The simple Donnan ratio

$$\frac{[H^{\cdot}]\,(\text{corpuscles})}{[H^{\cdot}]\,(\text{serum})} = \frac{[Cl^{\cdot}]\,\text{serum}}{[Cl^{\cdot}]\,\text{corpuscles}}$$

may therefore be erroneous

The Hasselbalch-Henderson equation is used in the calculation for the blood corpuscles in the form

$$pH = pK' - \log\frac{[BiK]}{[CO_2]},$$

where BiK is the bicarbonate concentration and K' a constant comprising the constants of the reaction and the activity coefficients It is quite possible in the corpuscles that pK' does not keep constant owing to the change in the composition of the protein phase with the changing pH Northrop and Kunitz[22] have pointed out that the protein phase has a greater effect on the activity coefficient of the cations than on the anions, and hence more effect on the hydrogen than on the bicarbonate ions

If the Hasselbalch-Henderson equation holds for the corpuscles as

for the serum, the gradual diminution of the difference of pH between corpuscles and serum follows from the values obtained for the CO_2 contents In the case of the calculations of Van Slyke, Wu, and McLean(26) where the concentrations have been worked out per 1000 grm of water in the protein phase, the same pK' is used for the corpuscles as for the serum, and the ratio

$$\frac{[H\ ]\ \text{corpuscles}}{[H\ ]\ \text{serum}} = \frac{[HCO_3']\ \text{serum}}{[HCO_3']\ \text{corpuscles}}$$

must hold if their concentrations are true activity measures This view is modified in the paper of Van Slyke, Hastings, Murray and Sendroy(27) The error in calculating the crystalloid ions per 1000 grm of water present is that the protein phase contains an unknown amount of water, and this amount may change as the pH changes, owing to changes in ionisation of the protein

A few calculations of the pK' in certain experiments have been made in the present experiments and a pK' of approximately 6 00 obtained, whilst Van Slyke, Wu and McLean(26) use 6 17 Van Slyke, Hastings, Murray and Sendroy(27) however obtain 5 97 by electrometric measurements on horse corpuscles The experiments with corpuscles using both the constant pressure and the constant volume apparatus of Van Slyke(28, 29) have not been very satisfactory as when acid is added to blood corpuscles a large amount of solid matter is deposited which makes the meniscus difficult to read and which also clings to the side of the apparatus, altering its volume The Barcroft apparatus where the measurements are done away from the reacting substances seems to give the best results in the case of corpuscles

As the difference in pH between the corpuscles and the serum does not diminish as the serum becomes more acid, pK' of the Hasselbalch-Henderson equation cannot be a constant The pH's of the corpuscles calculated from the equation are therefore erroneous

The fact that the pH's of the serum and the corpuscles do not approximate to the same value near the isoelectric point of hæmoglobin is another proof of the impermeability of the corpuscular wall to cations

In general the results given here show that the corpuscles and serum do not have the same reaction at any point, not even as the isoelectric point of the hæmoglobin is approached The corpuscles seem to buffer the plasma at the expense of themselves This is quite reasonable from a physiological standpoint as the cells in the body are in contact with the plasma and not the corpuscles

In the case of goose blood there is a possibility that an interchange

of ions takes place between the nuclei and the cytoplasm of the cells This exchange must be small if it takes place, or the pH of the corpuscles would vary in a very different manner from non-nucleated corpuscles The hydrogen ion concentration of the nucleated goose corpuscles is just as dependent upon the external conditions as is the case with non-nucleated corpuscles The functions of the nuclei are not clear but do not seem to be of much effect in the utilisation of oxygen and the expiration of CO_2 by the animal

The addition of cyanide to the blood may affect the nuclei of the corpuscles in such a way that their functions are destroyed entirely, and the corpuscles become the same in properties as non-nucleated corpuscles This is unlikely, but must be recognised as a possibility when discussing the results

SUMMARY

(1) A method is given of obtaining the true electrometric activity of the hydrogen-ions in oxygenated blood corpuscles

(2) In human blood the corpuscles are considerably more acid than the serum over the whole of the physiological range The pH of the corpuscles varies from 7 47 to 6 95 whilst the pH of the corresponding serum varies from 7 70 to 7 26

(3) The hydrogen-ion activity of goose blood corpuscles has been determined. The pH of the corpuscles varies from 7 59 to 7 08, whilst the pH of the corresponding serum varies from 7 84 to 7 37

(4) At the same CO_2 pressure goose blood appears to be slightly more alkaline than the human blood.

(5) The similarity between the shapes of the curves for goose blood and human blood indicate that the buffering mechanism is the same in both cases The nuclei of the bird's corpuscles do not seem to have much effect upon the buffering of the blood.

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THE EFFECT UPON THE THRESHOLD FOR NERVOUS EXCITATION OF THE LENGTH OF NERVE EXPOSED, AND THE ANGLE BETWEEN CURRENT AND NERVE

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THE EFFECT OF INTERPOLAR LENGTH UPON NERVOUS EXCITATION

Introduction It is well known that the minimum current required to excite a nerve decreases when the distance between the electrodes is increased Various explanations have been proposed in the past, but since most of the work was done some fifty years ago, they are somewhat unsatisfactory from the present standpoint

The general ideas of nervous activity then in vogue have since been modified and made more precise by two conceptions in particular

First the work of Lucas and others distinguished between the two forms of activity, "the local excitatory process," and "the propagated disturbance" The threshold for excitation was shown to depend upon purely local phenomena, and the remoter parts of the nerve had no other effect on the success or failure of a stimulus than sometimes to block the propagated disturbance which had already been initiated

Second, the ionic hypothesis elucidated the nature of the passage of electricity in media such as those of which the tissues are composed, and showed that any change which was brought about must occur through the action of ions

Nernst(1) applied this concept to the problem of nervous excitability, and, assuming that movement of ions *per se* could have no effect, he was immediately brought to his membrane hypothesis According to this a stimulating current acts only through the changes in ionic concentration which are brought about, and such changes can only occur in the neighbourhood of something which opposes the flow of ions, *e g* a semi-permeable membrane

Nernst made the further assumption that not only did the local excitatory process depend upon the ionic concentration, but that this concentration was always the limiting factor in excitation He therefore

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always kept fixed, and was the cathode The anode was movable The interpolar length was read off directly from a scale (not shown in diagram)
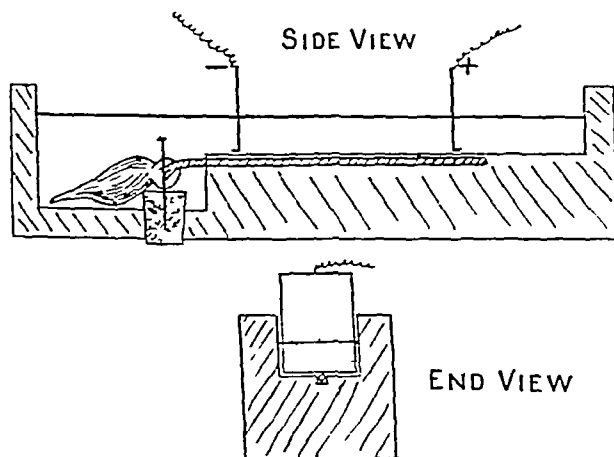

Fig 1 Apparatus for strength length determination.

It was assumed that there was a uniform potential gradient in the fluid from one electrode to the other, and that each extrapolar region was equipotential

The currents used were constant currents lasting about 0002 sec , hence the polarisation at the electrodes was not excessive Moreover, it was not cumulative, since the electrodes were connected by a short circuit between each observation, and hence would dissipate any charge they might have acquired

Experiment A frog's sciatic-gastrocnemius preparation was used The nerve was ligatured near the cord, and cut through central to the ligature The preparation was then set up in the apparatus as described, and left for about an hour to attain a steady state

The duration of the current was controlled by a Lucas pendulum and the strength by leading off from a low resistance potentiometer wire Two strengths were recorded usually differing by 5 p c , the upper of which caused a perceptible twitch of the muscle whilst the lower did not The results are recorded graphically as the line joining the two points corresponding to these two strengths

The experiment was performed with the interpolar length being made gradually shorter, then it was repeated with the length increasing, and finally the first was again repeated

concluded that if the requisite concentration was attained, excitation would certainly occur (this assumption was modified a little to account for the results of stimulation with currents of long duration)

Nernst's theory is well supported by the results of stimulating a nerve with constant currents of various durations, and with alternating currents of various frequencies   Except when the time intervals are great, the value of the minimum exciting current lies very close to that calculated from the above assumptions

The advantage of a theory such as Nernst's is obvious, for it allows a theoretical relation to be worked out in terms of the known properties of ions   The assumptions, moreover, are simple and precise

Since Nernst was not concerned with changes in the position of the electrodes, it was unnecessary to make any assumptions as to the extent of membrane against which the ions had to be concentrated   For the purpose of the present investigation, however, the following assumption on this point will be made

"Excitation will occur at any point in the membrane, when the ionic concentration at that point attains a certain value"

It is the object of this paper to see to what extent Nernst's theory is able to explain the variations of excitability consequent upon changes of interpolar length

Variation of threshold with interpolar length   It has long been known that a current is more efficacious the longer the stretch of nerve it traverses   This is entirely confirmed by the following observations, which are in complete agreement with the older work, and have the advantage that they were obtained by a method which avoids any manipulation of the nerve during the experiment

Apparatus   An ebonite trough filled with Ringer's fluid was used, of the following construction (Fig 1)

The trough was deep at one end to hold the muscle, and a cork was provided to receive the pin through the knee   This deep part was 3 cm in length   The rest of the trough, 6 cm in length, was shallower by about the thickness of the knee, so that the upper surface of the knee was just above the floor of the shallow part   The centre of this floor was grooved by a longitudinal slot, of such a size that it would just easily contain the nerve   When the preparation was set up, therefore, the nerve lay in the groove and passed horizontally to the knee, and the muscle lay below in the deep part of the trough

The electrodes were plane vertical plates of bright silver, which fitted exactly the section of the trough   The plate near the muscle was

always kept fixed, and was the cathode The anode was movable The interpolar length was read off directly from a scale (not shown in diagram)
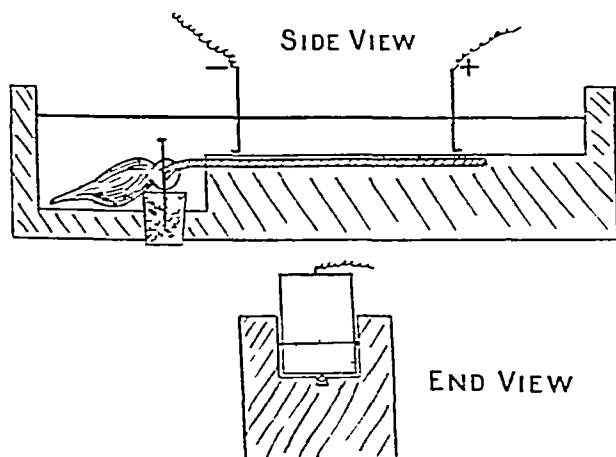

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The experiment was performed with the interpolar length being made gradually shorter, then it was repeated with the length increasing, and finally the first was again repeated

If these three did not on the whole agree to 10 p c , they were all rejected without any further analysis In the other event all three were accepted and no further rejection was done As a matter of fact the apparatus was found reliable, and after the technique had been acquired no rejections were necessary

Results It was found that the longer the interpolar length the more excitable the nerve up to a point, but that after 20 mm , an increase to 40 mm had no further favourable effect

Taking the strength of this lower limit as two units (for reasons which will appear later), the relation between length and threshold is fairly constant from nerve to nerve, and if we plot the threshold in these units against the length in arbitrary units which differ slightly from nerve to nerve, the results of all the experiments can be made to lie on the same curve (Fig 2)
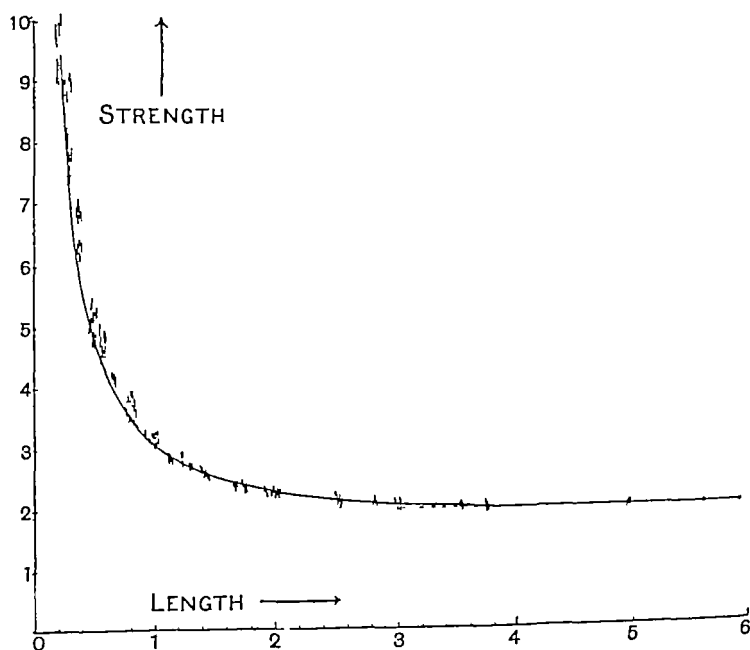

Fig 2   Strength length relation. Lengths measured in "analytical units" of about 5 mm. Equation of curve, $y = 1 + \coth x/2$

Significance of strength-length relation If the ineffectiveness of the current flowing through a short length is to be explained in terms of ionic movement, there are two possibilities

(1) The electric field must be inadequate to concentrate the ions much when the length is short

(2) The back diffusion must be too great to allow of much concentration

These two cases are not mutually exclusive, and that the second certainly is in operation is seen from the effect of interpolar length upon the chronaxie

It has been shown by many workers in France (2) that the chronaxie becomes less as the interpolar length is diminished, and this is confirmed by one or two experiments with the above apparatus, where it was found that the strength-length curve was to some extent changed when the duration of current flow was altered   It is impossible, however, to estimate whether the alteration of back diffusion is sufficient to account for the change in threshold unless a definite conception of the exact movement of ions is assumed   If, however, it is assumed that the change of threshold is mainly dependent upon changes in the electric field, this assumption may be given a plausible basis, and be made to yield a quantitative explanation

Dependence of electric field upon interpolar length   Hermann (3) and Hering working on the question of internal polarisation in nerves came to the conclusion that their phenomena could be explained on the assumption that the medullated nerve fibre was a cylinder with a resistant sheath and a conducting core

Hermann made a model nerve with a platinum wire as core, and with a relatively resistant $ZnSO_4$ solution for the sheath

Hering's model was a piece of grass soaked in saline, the solution being the core, and the grass envelope the resistant sheath

There is obviously nothing like metallic conduction in a nerve so that Hering's model is a more accurate representation, but both models realised the phenomena of internal polarisation, for both satisfied the essential condition of a resistant sheath and a conducting core   If this model is accepted, then, the membranes responsible for internal polarisation are cylinders co-axial with the nerve

It will be further assumed that these membranes are also the ones which are responsible for nervous excitation on Nernst's theory

Nernst himself specified only that the membrane might be considered at an infinite distance from any other   Hill (4) on the other hand worked out a modification wherein two membranes were assumed close together   But neither investigator assigned to the membranes any definite position in the structure of the nerve

The alternative suggestion that the membranes are placed transversely is not supported by any histological evidence, and is difficult to reconcile with the classical observation that at "make," the excitation is localised at the cathode

The inability of transverse currents to excite will be considered later in this paper

Accepting therefore the above assumptions, it will be possible to calculate the distribution of current in the nerve when it is placed in an electric field, and hence to estimate the force concentrating ions against the cylindrical membrane

It must be added that the theoretical treatment which follows does not claim to be more than a first approximation, suitable only to explain the observations which are under discussion   It is hoped that it will be possible later to develop it to embrace a wider range of phenomena

MATHEMATICAL TREATMENT OF RESISTANCE OF NERVE

Definitions   The nerve fibre is assumed to be a cylinder with a relatively well-conducting core, and a relatively non-conducting sheath The current is therefore supposed to enter radially through the sheath, and then to pass longitudinally up the core

It is assumed that all points in the system take up a steady potential immediately the circuit is closed

Now the resistance of the core to longitudinal currents varies directly as its length, whereas the resistance of the sheath to radial currents varies inversely as the length of the sheath through which the current passes   There must therefore be a certain unique length l, such that the longitudinal resistance of l mm of core = the radial resistance of l mm of sheath

For the purposes of the mathematical analysis which follows, it is convenient to measure all lengths in terms of this length l as unit length, and similarly to measure resistance in terms of the resistance of l mm of core as unit resistance

It is not possible to measure l directly, but it will be shown that the value can be deduced from the strength-length curves

Expressed in these units, let

x = lengths measured along axis of nerve,

U = potential applied to outside of sheath at any point x

V = potential of core at any point x

The problem is assumed to be radially symmetrical

The system is defined as follows

The potential U is 0   from $x = 0$ to $x = a$

$$\mathfrak{t}x \qquad x = a \qquad x = a - s$$
$$\mathfrak{t}s \qquad x = a - s \qquad x = a - s - b$$

The two extremities $x = 0$, $x = a - s - b$ are supposed to be closed by transverse membranes of resistance $\tanh \alpha$, $\tanh \beta$ respectively according as < 1, $\coth \alpha$, $\coth \beta$ respectively, according as > 1

Consider first the part from $x = a$ to $x = a - s$

The current leaving the core at any point $= \partial^2 V / \partial x^2$ but also $= V - \mathfrak{t}x$

The solution of this differential equation is

$$V = A \cosh x - B \sinh x - \mathfrak{t}x$$

as may easily be verified.

It is readily seen that $\partial^2 V / \partial x^2$ may be expressed as $l \sinh \overline{x - c}$ where l, c are constants

For since $\partial^2 V / \partial x^2$ is positive at the anode and negative at the cathode and is also a continuous function of x between these two limits, it follows that at some point c intermediate $\partial^2 V / \partial x^2 = 0$

$$B \cosh c \, [A/B - \tanh c] = 0$$

And since $B \cosh c \neq 0$

$$A = l \sinh (- c)$$
$$B = l \cosh (- c)$$

$$\partial^2 V / \partial x^2 = l \cosh x \sinh (- c) - l \sinh x \cosh (- c) = l \sinh \overline{x - c}$$

Thus since $\sinh \overline{x - c}$ increases with x for all values of x, $\partial^2 V / \partial x^2$ has a greater value at the cathodic extremity than at any other point in the range over which the equation holds (i e from a to $a - s$)

But since the membranes are assumed to be longitudinal $\partial^2 V / \partial x^2$, which is the current leaving the core, is the current concentrating cations against them.

Thus cations will most be concentrated at the cathodic extremity of the range, and the excitator value of the current is given by

$$(\partial^2 V / \partial x^2)_a = A \cosh a - B \sinh a$$

Now introduce a new variable ψ defined as follows

$$\frac{\partial \cdot V}{\partial x^2} \Big/ \frac{\partial V}{\partial x} = \tanh \psi \text{ according as } < 1, \ \coth \psi \text{ according as } > 1$$

Then since $\partial^2 V/\partial x^2$, $\partial V/\partial x$ are both continuous functions of x whatever continuous function U may be, ψ will also be a continuous function

In the present case

$$\tanh \psi = \frac{A \cosh x + B \sinh x}{A \sinh x + B \cosh x + v} \text{ between } x = a, \ x = a + s$$

If ψ_a, ψ_{a+s} are the values which ψ assumes when $x = a, \ a + s$ respectively we may write the two corresponding equations and solve for A and B

Then substituting these values in

$$(\partial^2 V/\partial x^2)_a = A \cosh a + B \sinh a$$

we obtain $\qquad \left(\dfrac{\partial^2 V}{\partial x^2}\right)_a = v \sinh \psi_a \ \dfrac{\sinh \overline{s - \psi_{a+s}} + \sinh \psi_{s+s}}{\sinh \overline{s + \psi_a - \psi_{a+s}}}$

Now the regions from $x = 0$ to $x = a$, $x = a + s$ to $x = a + s + b$ are particular cases of stretches exposed to constant potential gradients, namely, when the gradient is zero

Hence

$$\tanh \psi = \frac{A_1 \cosh x + B_1 \sinh x}{A_1 \sinh x + B_1 \cosh x} = \frac{A_1/B_1 + \tanh x}{A_1/B_1 \tanh x + 1} = \tanh \overline{x + c}$$

when $\tanh c = A_1/B_1$

$\psi - x = c$, a constant over the whole range

$\psi_0 - 0 = \psi_a - a$

Also $\psi_{a+s} - (a + s) = \psi_{a+s+b} - (a + s + b)$

Now

$\tanh \alpha = \text{resistance of end at } x = 0 = \left(\dfrac{\partial V}{\partial x^2} \Big/ \dfrac{\partial V}{\partial x}\right)_0 = \tanh \psi_0,$
$$\alpha = \psi_0$$

$\tanh \beta = \text{resistance of end at } x = a + s + b = - \left(\dfrac{\partial V}{\partial x^2} \Big/ \dfrac{\partial V}{\partial x}\right)_{a+s+b}$
$= - \tanh \psi_{a+s+b},$ $\qquad\qquad \beta = - \psi_{a+s+b}$

Substituting these values in the above two equations

$$\psi_a = (a + \alpha),$$
$$\psi_{a+s} = - (b + \beta)$$

These may now be substituted in the equation for $(\partial^2 V/\partial x^3)_a$ giving

$$\left(\frac{\partial^2 V}{\partial x^2}\right)_a = \iota \sinh \overline{a + \alpha} \ \frac{\sinh \overline{s + b + \beta} - \sinh \overline{b + \beta}}{\sinh \overline{s + a + \alpha + b + \beta}}$$

This formula holds provided that $\tanh \alpha$, $\tanh \beta$ obtain

If coth α obtains each term containing α becomes cosh instead of sinh, if coth β obtains each term containing β becomes cosh instead of sinh, if coth α and coth β obtain each term containing α and β remains sinh, others become cosh

Application to strength-length curve The interpolar length is represented by the length s which is exposed to the constant potential gradient, the extrapolar lengths being the parts in the equipotential solutions

We have seen that the current concentrating ions against a longitudinal membrane is given by the value of $\partial^2 V/\partial x^2$ and that this continually increases from one end to the other of the interpolar stretch It may also easily be shown that the values in the extrapolar regions are always intermediate between the two extremes of the interpolar stretch, hence, according to the assumption at the beginning of the paper, excitation should occur at the cathodic extremity of the interpolar stretch On similar assumptions but supposing the membranes to be transverse, excitation should occur approximately in the centre of this stretch, where $\partial^2 V/\partial x^2$ vanishes

Experiments with the local application of weak alcohol showed that excitability was only affected when the application was at the cathodic extremity of the interpolar stretch

Now since the interpolar length was in all cases altered by moving the anode,

"a," the cathodic extrapolar stretch remains constant

Hence $s + b = \text{constant} = c$

$$\left(\frac{\partial V}{\partial x}\right)_a = v \sinh \overline{a + a} \ \frac{\sinh \overline{\beta + c} - \sinh \overline{\beta + c - s}}{\sinh \overline{a + a + \beta + c}} \text{ or cosh, etc}$$

Now if $c > 6$ we may convert these hyperbolic functions into their exponential equivalents and obtain

$$(\partial^2 V/\partial x^2)_a = (1 \pm e^{-2(a+a)})(1 - e^{-s}) v/2$$

correct to less than 1 p c , or, regarding $(\partial^2 V/\partial x^2)_a$ as attaining a constant value whenever the threshold is just reached,

$$v \propto 1/(1 - e^{-s}),$$
$$\propto 1 + \coth(s/2)$$

The experimental results when plotted at the scale of about 5 mm to the analytical unit fit this equation well And since $c > 30$ mm $c > 6$ units and the above condition is realised

Method of finding value of analytical unit in each case.

The threshold potential gradient corresponding to each interpolar length is expressed in units such that the value corresponding to infinite length $= 2$

The curve $y = 1 + \coth x/2$ is drawn. y is then made equal to each of the threshold potential gradients in turn, and the corresponding value of x is read off.

Thus to each potential gradient there corresponds on the one hand the observed interpolar length in mm., and on the other the derived value of x.

These latter two quantities when plotted against each other are found to lie upon a straight line which passes through the origin. The gradient is easily seen to be the analytical unit of length expressed in mm.

COMPARISON WITH EXPERIMENTAL RESULTS

The conclusion of the mathematical treatment shows that the strength-length curve, expressed in units such that the minimum threshold strength was 2, is a curve involving a single arbitrary constant —the value of the analytical unit of length. If all nerves had the same unit, all should have the same strength-length curve, actually, however, we should expect that there would be a slight variation from nerve to nerve. But when the lengths (in mm.) were divided each by their own unit, we should expect that they all fell upon the same curve.

This has already been quoted as fact when considering the experimental results.

Furthermore, the curve in question should coincide with

$$v \propto 1 + \coth (s/2)$$

That this is the case with considerable accuracy may be seen from Fig 2. The curve here is that corresponding to the above equation, and the experimental lines are seen to fit it within the general limits of the experiment. The lengths are plotted in analytical units, and the strengths in units such that the minimum is two.

It therefore appears justifiable to regard the strength-length curve as due largely to the variation in current distribution consequent upon the resistant sheath of each fibre.

The change of chronaxie with different lengths shows that the rate of dissipation must play some part in altering the threshold, but this part need not be very great since the strength-length curve is capable of being explained quantitatively without reference to it.

COMPARISON WITH FORMER WORKERS

MARCUSE(5)   The first results that I know of which can be studied quantitatively are those of Marcuse. In principle his apparatus was similar to mine, but the extrapolar regions of the nerve were lifted out of the solution. This must involve errors due to handling, and the meniscus makes it impossible to read the interpolar length to an accuracy greater than 1 or 2 mm.

Fig 3 shows my theoretical curve and all the results of Marcuse where the cathode was kept fixed   Those where the cathode was moved would include errors from variations of local excitability from point to
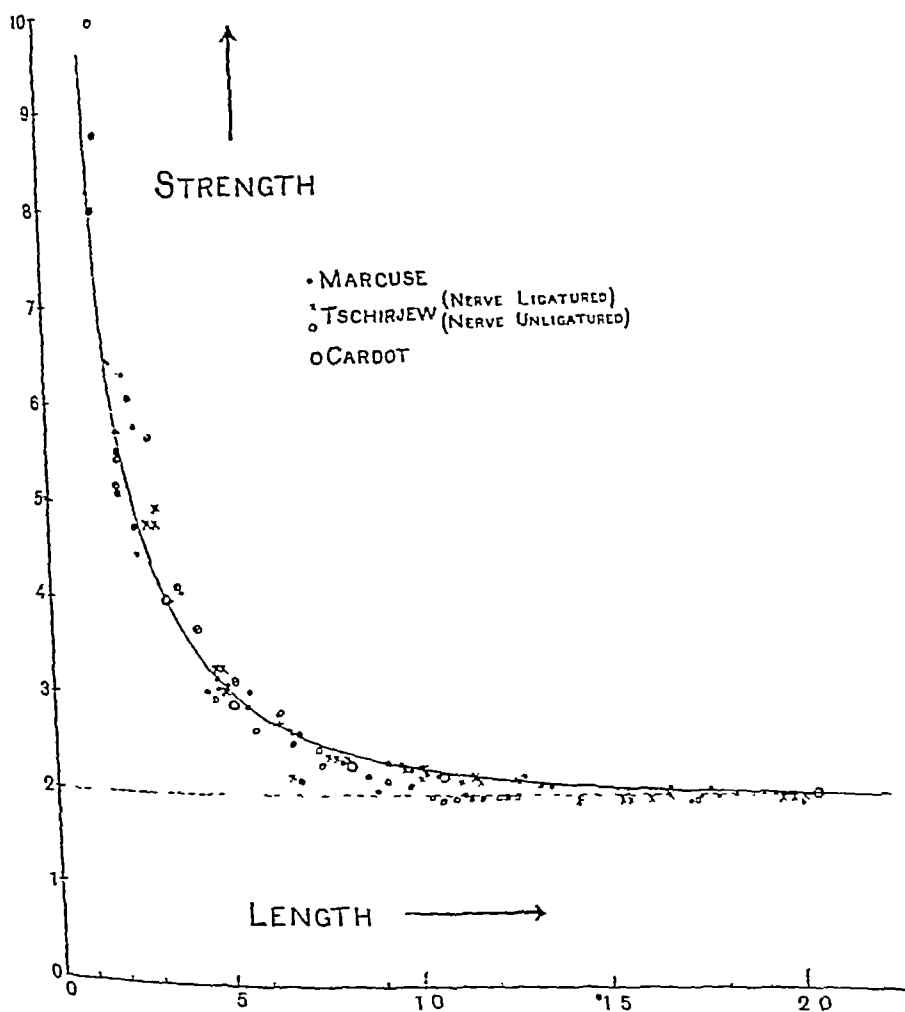

Fig. 3 Strength length relation. Results of former workers   Units as in Fig 2.

point, besides the deviation due to the fact that the theoretical curve for this case is not the same as for the former

The results given show a fair agreement with the theory, and suggest a fairly large random error, but no consistent divergence

Marcuse found that some of his curves exhibited a minimum threshold corresponding to a certain length lengths greater than this required a higher threshold He thought that those curves which did not show this would do so were the length sufficiently increased However, if we consider only the results with cathode fixed this minimum is not much in evidence, and, as has been mentioned, the other results are not strictly comparable

Tschirjew's(6) results were published in the same year as Marcuse's, and apparently quite independently

His method was quite different Electrodes were applied to the nerve in air, and the interpolar length varied by two methods

(1) The electrodes were moved

(2) The nerve was ligatured in the interpolar region, and the whole nerve was moved across the electrodes

Thus, though the length of nerve substance between the poles was constant, the length of nerve between the ligature and the electrode nearer the muscle was varied

These results when plotted are seen to agree with the theoretical curve about as well as those of Marcuse

Tschirjew's formula $v = a + b/s^m$ where a, b, m are constants is one which would fit any curve of this type within the rather wide limits of error It has one more arbitrary constant than mine, has no theoretical basis, and hence need not be considered

Cardot(2) Comparatively recently the same investigation has been carried out by Cardot Electrodes were applied to the nerve in air, and the interpolar length altered by moving the anode A large resistance was placed in series with the nerve Unfortunately he only gives one result, but that is seen to fit excellently the theoretical curve

To summarise the results of these workers

The large variations of Marcuse and Tschirjew allow of no conclusion other than that the results are not inconsistent with the theory

Cardot's curve may be taken as confirmatory

VARIATION OF THRESHOLD WITH ANGLE BETWEEN CURRENT AND NERVE

The foregoing results have been explained on the assumption that excitation is brought about by the concentration of ions against a membrane which is cylindrical and co-axial with the nerve A few *a priori* reasons in favour of this assumption have been given, and it is now necessary to deal with what appears to be a serious objection

From the time of Galvani onwards it has been recognised that a

nerve is nearly or quite inexcitable to currents perpendicular to the axis This seems to favour the assumption of a transverse membrane, for at first sight it would appear that the transverse direction would be the most efficient for concentrating ions against a longitudinal membrane

Determinations were made on the relation between threshold and angle between current and nerve, and the results are in agreement with previous workers These experiments were all completed before the development of the foregoing mathematical theory with which they are in close accord

Apparatus A frog's sciatic-gastrocnemius preparation was used The nerve was ligatured near the cord, and cut through central to the ligature It was stretched horizontally in a trough of Ringer's fluid, through which a current was passed between two non-polarisable Ag-AgCl plates Seen from above, the trough was square, and the plates occupied the whole of two opposite sides

The lines of flow of the current were therefore all parallel to each other, and perpendicular to the two plates It is this direction which is referred to as the direction of the current, though the actual direction in the nerve is different
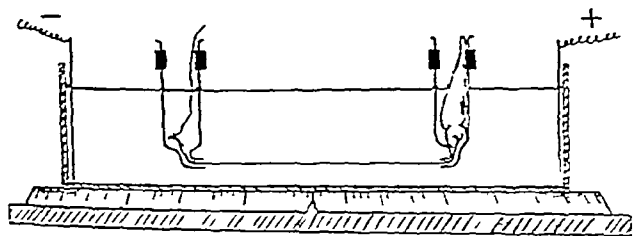

Fig 4 Apparatus for determining relation between threshold and angle

The trough and electrodes were mounted on a small turn-table graduated in degrees

Both ends of the nerve entered the capillary ends of two tubes, and the muscle rested in the wider body of one of them A screw controlled the distance between the tubes by operating upon the arms which held them. These arms were attached to a rigid stand so that they remained fixed when the turn-table rotated By this means the angle between current and nerve was varied

The advantages of holding the nerve in this way are

(1) The portion of nerve exposed to the current is confined to the part between the tubes

(2) This length may be varied accurately and easily

(3) The nerve is entirely straight in the exposed part

Proof of (1) is derived from three sources

(*a*) If current entered it would have to leave by the same opening

(*b*) The strength-duration curve shows no *a* curve

(*c*) If the nerve is completely withdrawn into the tube with the muscle no contraction can be produced

Experiment A frog's sciatic-gastrocnemius preparation was set up in the tubes as described, and was left for about a quarter of an hour to attain a steady state before the determinations were made

The stimulus was in all cases a constant current usually of about 002 sec duration delivered by a Lucas pendulum, the strength was controlled by leading off from a low resistance potentiometer wire

At every 10° in the whole revolution, measurements were taken of the least potential led off which would bring about a perceptible contraction of the muscle The limits of experimental error are recorded as in the strength-length determinations

In order that the readings of the turn-table should give the angle between the current and the nerve, it was necessary that the nerve should be set parallel to the plates when they were set at an angle 90° In the earlier experiments this was done by eye, but later silver wires were inserted into the mouths of the two tubes and each connected to a terminal of a sensitive galvanometer The current was then passed between the plates of the trough, and the tubes adjusted to give no deflection in the galvanometer Their openings are now in a line perpendicular to the lines of flow

Results From the first it was clear that the excitability was roughly proportional to the cosine of the angle between current and nerve It was therefore thought well to plot the results in polar co-ordinates, for, not only is a cosine relationship represented by this method as a straight line, but also any zero error which there might be will do no more than rotate the graph about the centre through an angle equal to this error

The following convention is therefore adopted

If the current is flowing through the nerve in the direction *AB*, draw from the origin, *O* a line parallel to *AB* And if the strength of the current necessary to excite is 38 units, then draw the line *OP* 38 mm long The point *P* will
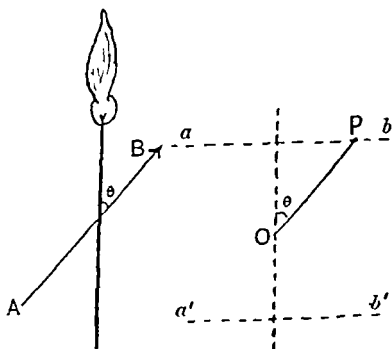
therefore represent the two related variables, the strength of current given by the distance OP and the direction given by θ, the angle which OP makes with the direction of the nerve
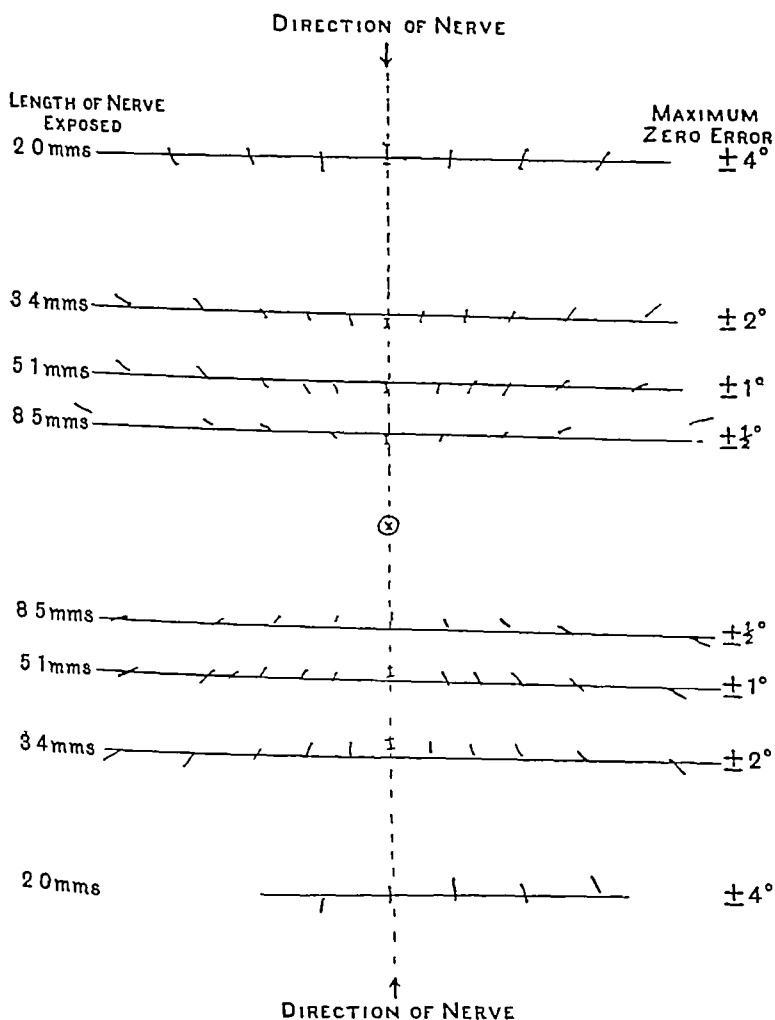

Fig 5   Relation between threshold and the angle which the current makes with the nerve

If then the length OP is always inversely proportional to the cosine of the angle it makes with the nerve, P must always lie on a straight line perpendicular to the direction of the nerve (as ab, $a'b'$)

The results show that this is very nearly the case   The actual figure

(Fig 5) gives several experiments performed upon different lengths of nerve from the same frog

Some experiments give closer approximations to straight lines, but the above is typical

The divergence of the results from two straight lines perpendicular to the direction of the nerve seems to be of three types

(1) The lines were sometimes straight and parallel but not perpendicular This is what would be expected as the result of zero error It was found most pronounced in the earlier experiments when the zero was set by eye In the later ones, where the galvanometer was used the error was never greater than that recorded beforehand as the maximum possible error from the galvanometric reading

(2) In the earliest experiments it was often found that the lines were not straight, but markedly concave towards the origin

Experiments directed to the investigation of this phenomenon showed that it was not caused by skewness of the tubes holding the nerve nor by any sort of progressive change taking place throughout the course of the experiment

It was noticed in a preparation which showed the phenomenon most markedly, that the nerve was hanging loosely between the tubes, and that it was bent somewhat at the place where the branch to the hamstring muscles leaves the sciatic trunk, so that the two halves of the exposed nerve were in slightly different directions

To see whether this was the cause the following experiment was performed (Fig 6)
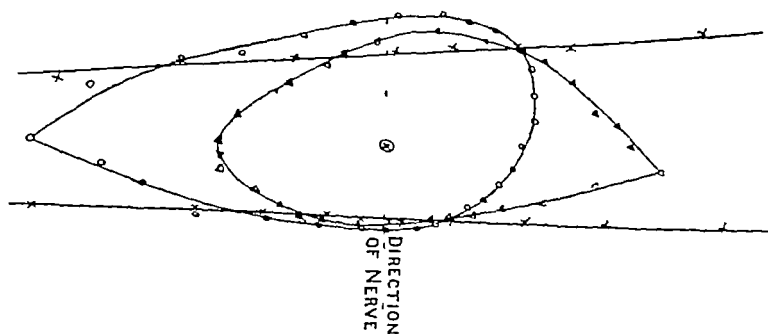

Fig 6   Effect of bending nerve

A preparation was set up slightly stretched so that there should be no appreciable bend, and a set of determinations made. The results gave straight lines as shown by the crosses. Then the nerve was artificially bent by pulling the centre to one side. The determination gave results very similar to those obtained accidentally before, the main features were that the two branches of the curves were concave towards the origin, and met in a rounded curve at one end and a point at the other, as shown by circles. The nerve was then bent the other way and the results showed that the round and pointed ends had been reversed (triangles). It was therefore concluded that the concavity towards the origin when present was caused by the bending of the nerve.

This conclusion was confirmed by all the subsequent experiments, in these the nerve was kept slightly stretched, and this concavity never appeared.

This source of error is of importance in criticising the earlier work on this subject, for it was never expressly mentioned, and, I believe, not recognised by those whose results showed this concavity towards the origin.

(3) The later results, which were free from these two errors, were usually not quite straight, but slightly convex towards the origin. The effect seemed to be diminished or abolished when special precautions were taken to ensure that the nerve lay in the centre of the capillary tubes.

In any case this error in most cases only just exceeds the experimental error, and it will probably not be far wrong to conclude that the component of the current in the direction of the nerve is alone effective as a stimulus. The component at right angles has practically no effect, but such as it has seems to be *inhibitory*.

Independence of cosine relation on length of nerve or duration of current

In order to be the more certain of the generality of the cosine relation, it was thought well to show that this relation was not dependent upon the particular length of nerve exposed to the current, or the particular duration of current employed.

Length The lengths used for the previous determinations varied over the range of 2 mm to 20 mm, and, as is clear from the results shown, though the actual values of excitability are greatly changed by variations of length, the cosine relation is unaffected.

Duration To find what effect other durations of current have upon this relation it would have been possible to repeat the former experiments, using currents of different durations.

The procedure actually adopted, however, was to find the strength-duration curve at different angles between nerve and current

The curve was determined after the method of L u c a s A given length of nerve was taken, and set parallel to the current, and the strength-duration curve was obtained Then the curve was repeated at one or two other angles Finally the first curve was repeated

If each of the strength-duration curves is reduced so that the strength at infinite duration is 1, then all the curves coincide remarkably well In fact, there was never a greater divergence between the first curve and any subsequent one, than there was between it and its repetition at the end of the experiment

Significance of the cosine relation From the foregoing results it seems justifiable to conclude that d u B o i s R e y m o n d was correct in his suggestion that the threshold was inversely proportional to the cosine of the angle between current and nerve, and it appears that the transverse component is ineffective no matter what the length of nerve exposed, nor what the duration of current employed

Now we have already seen the way in which threshold is related to interpolar length, and it is clear that if transverse stimulation is equivalent to an interpolar length of the order of the diameter of a nerve fibre, then the strongest currents which were used would be quite inadequate

This statement may be made quantitative on the lines of the foregoing mathematical treatment

We have already seen in the case of stimulation with longitudinal currents that v, the threshold potential gradient, and $\partial^2 V/\partial x^2$, the corresponding potential difference across the sheath, are related by

$$\left(\frac{\partial^2 V}{\partial x^2}\right)_a = v \sinh \overline{\alpha + a} \ \ \frac{\sinh \overline{\beta + b + s} - \sinh \overline{\beta + b}}{\sinh \overline{\alpha + a + \beta + b + s}} \ \text{or} \ \cosh, \text{etc},$$

or when $s = 6$ $= (1 \pm e^{-2(\alpha + a)}) (1 - e^{-s}) v/2,$

or when $a > 1$ $= \text{order of } v/2$

Thus, if a transverse current is to be effective as a stimulus, the applied potential gradient v' must be at least sufficient to produce a potential difference across some point in the sheath equal to $v/2$

Now if the external diameter of a nerve fibre is $2r$, at no point in the sheath is the potential difference across it $> v'r$

Therefore the minimum value of v' is defined by

$$v'r = v/2$$

$v'/v = 1/2r$ where r is measured in analytical units

$\qquad = 5/2r$ where r is measured in mm

Now $r = $ order of 8/1000 mm $\qquad v'/v = $ order of 300

We thus conclude that the transverse current is ineffective because, to produce the same ionic concentration as a longitudinal current, it needs to be more than 300 times as strong

The strongest currents used in my experiments were far weaker than this

COMPARISON WITH FORMER WORKERS ON VARIATION OF EXCITABILITY WITH ANGLE

(1) FICK(7) He investigated the relation over the range from 34° to 90° by a method which depended upon the nerve dipping into a solution through which a current was passing in parallel lines   The results were in very good agreement with the cosine relation and FICK concluded that this law described them   He found, however, that a transverse current was always capable of exciting, though the strength required was much greater than any used in my experiments

The explanation of this is probably due to the fact that the nerve, supported at two ends, looped down into the solution   It therefore behaved as in my experiments when the nerve was not slightly stretched As has been described, this condition gives rise to results which diverge from straight lines by being concave to the origin   This is the very divergence from the cosine relation which FICK obtained

(2) ALBRECHT and MEYER(8)   These workers did not study the relation at various angles but contented themselves with finding whether a nerve can be excited by a strictly transverse current

They confirmed the above speculation by showing that when great precaution is taken that the current shall be perpendicular to the nerve at all points, the nerve is quite inexcitable

(3) TSCHIRJEW(6)   The results of this worker are in complete disagreement with those of all others   This is probably due again to the failure of the precaution to keep the nerve stretched straight   When plotted in polar coordinates these curves strongly suggest badly bent nerves, and since his nerve was tied by threads to the surface of a glass plate, the extremities would probably be lying in any direction, and this would account for the results

These results, then, appear to be valueless, and on the whole the earlier work confirms the cosine relation

The theoretical interpretation of the cosine relation is well summed up by Hermann in his *Handbuch* He here puts forward three reasons why it is useless to try to establish a relation between excitability and angle

(1) The nerve is not a homogeneous conductor, and hence the distribution of current in it will vary with angle

(2) The transverse resistance is greater than the longitudinal, and hence the nerve will appear more excitable to longitudinal currents

(3) To compare longitudinal and transverse excitability, equal lengths of nerves must be taken, hence in the longitudinal case a length of 01 mm must be used

But these objections are only valid if no attempt is made to compensate for the various factors In the present work, however, each of these is taken into account, and, instead of them rendering the results valueless, they are at the basis of the explanation

The inefficacy of a transverse current has been shown to be due to the great resistance which a nerve offers to the passage of a current in a transverse direction So obvious a possibility was not overlooked by the earlier workers, but definitely rejected by them owing to an error which seems to have been peculiarly widespread

Hermann placed a large number of nerves parallel to each other and found the electrical conductivity of the mass when a current was passed parallel to the fibres, and when perpendicular to them

The conductivity in the former case was five times that in the latter

From this it appears to have been very generally concluded that the alteration of conductivity between the longitudinal and transverse directions will only account for an alteration of excitability of 1 5

This, however, is only valid if this ratio represents the variation of current at the place where excitation occurs

Now on the basis of the above calculations the conductivity of a single fibre to a transverse current is extremely small, and consequently in Hermann's experiment the conductivity measured would be merely that of the fluid between the fibres The results therefore give no information about the conductivity of the *fibres* themselves, and cannot be applied to this question

Summary

(1) The relation between threshold and interpolar length is determined, and the results are given in Fig 2

(2) The experimental results accord well with the theoretical curve calculated from the following assumptions

(*a*) The nerve is a cylinder with a resistant sheath and a conducting core.

(*b*) Nernst's assumptions regarding the dependence of excitation upon ionic concentration are accepted

(*c*) Excitation is brought about by the current leaving the nerve through the cylindrical sheath

(3) The relation between threshold and angle is determined and the former is found to vary inversely as the cosine of the angle between the current and nerve

(4) This cosine relation obtains for different lengths of nerve and different durations of current

(5) The cosine relation is directly deduced from the mathematics put forward to explain the relation between threshold and interpolar length

(6) Observations of former workers on these two relations are not inconsistent with the quantitative results of this explanation

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THE ACTION OF LIGHT ON THE EYE   Part I   The Discharge of Impulses in the Optic Nerve and its Relation to the Electric Changes in the Retina

By E D ADRIAN and RACHEL MATTHEWS

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INTRODUCTION

DURING the past two years the impulses occurring in various types of peripheral sense organ have been investigated by recording their action currents with the capillary electrometer and an amplifying system[1] It has been found that there is a close agreement in the behaviour of the different kinds of end organ and in the impulse discharges set up by them   The present work breaks new ground in that it deals with a special sense organ and records the impulses in fibres which differ to some extent from those of a peripheral nerve trunk   The work is far from complete, but it has already shown that the activity of the optic nerve is in close agreement with that of other sensory nerves   On the other hand, the action of the receptor apparatus of the eye is naturally far more complex than that of the peripheral sense organs

With the exception of some of Fröhlich's observations on the cephalopod eye[2] we have been unable to find any references to work on the action currents of the optic nerve produced either by exposure of the eye to light or by direct stimulation of the nerve   The retinal currents have been investigated repeatedly with increasing accuracy of technique since their discovery by Holmgren, and are a valuable index of the activity of the retina, but they are due to processes taking place in the eye rather than the optic nerve and they do not tell us how the results of the retinal activity are transmitted to the brain

The present work falls into three main sections   The first deals with the discharge of impulses in the optic nerve, the second deals with the relation of the optic nerve discharge to the retinal current, and the third with some points which concern the excitation of the retinal structures by light   An appendix by one of us (R K M) gives some data on the histology of the eel's optic nerve

Technique Preliminary experiments had shown that the recording of optic nerve impulses was a more difficult undertaking than the recording of impulses from the peripheral sense organs The difficulties all arise from the special structure of the nerve The intracranial part is delicate and easily damaged, but the chief trouble is due to the short length of the nerve and the large number of fibres in it The greater the number of fibres in a nerve and the smaller the distance between the leading off electrodes, the more difficult it will be to detect the electric response of a single fibre, yet if a record of sensory action currents is to give much information the activity must be confined to a few nerve fibres to allow the individual impulses to be distinguished

Limits of amplification

It might appear that these difficulties could be overcome by increasing the amplification of the action currents. The earlier work on sensory nerves had all been carried out with an electrical system just capable of showing a change of 01 millivolt lasting for ·001 sec. This work had left the impression that little was to be gained by an increase in sensitivity, but the point had never been tested and as the first step in the present research we added to the amplifier a fourth valve shielded in a separate case and provided with its own batteries The amplification with this arrangement was 25,000 but the base line was unsteady, showing constant small oscillations due to instability within the amplifier. A potential difference of 1 microvolt lasting for 001 sec. gave a deflection large enough to be detected in spite of the unsteady base line When the amplification was cut down to 5000 the oscillations due to the amplifier were scarcely appreciable, but a new set of small oscillations appeared if a portion of the frog s sciatic was included in the input circuit. These were changes of potential of the order of 1–2 microvolts The slightest drying of the nerve increased them enormously and constant irrigation reduced them. It is possible that they might be reduced still more by a more perfectly balanced Ringer's fluid, but it is clear that for the present at least these fluctuations set the limit to the useful amplification which can be employed. If the action current does not produce a potential difference of at least three or four microvolts, it will have little chance of showing up against the unsteady background and as this unsteadiness is due to the tissue itself it cannot be overcome by the most perfect amplifying system. With our present electrometer there has never been any advantage in increasing the amplification above 5000 and we have usually employed three valves only (× 1750)

Preparation Our earliest experiments were made on the optic nerve of the frog (*R temporaria*) They were successful in showing that action currents could be detected in the nerve when a light was thrown on the eye but it was quite clear that some more suitable preparation would have to be found if we were to learn much from the records The ideal preparation would provide a long optic nerve with not many fibres, we were anxious to work on vertebrate eyes and anxious to avoid the complications entailed by a warm-blooded mammalian preparation All these conditions are satisfied by eye and optic nerve preparations from

various fish and most of our experiments have been carried out on preparations from the eel, *Conger vulgaris* Live Conger eels happen to be more easily obtainable in Cambridge than fresh water eels (*Anguilla*), and they remain in good condition for many days if kept in a large tank of aerated sea water In a specimen weighing 3 lbs the optic nerve is about 15 mm long and thinner than a frog's sciatic

The retina has the normal structure of the teleost eye (3) containing both rods and cones and differing little from the mammalian retina except in the presence of the campanula and the absence of anything corresponding to the fovea The number of fibres in the optic nerve is about 10,000.

In most of the experiments the eel was decapitated, the brain pithed, and eye and optic nerve removed from the head In a few experiments the brain and spinal cord were destroyed, but the eye was left *in situ* with its circulation intact and leads were taken from the intracranial part of the nerve During the dissection the eye was shielded from the light by a small cap of black fabric

As a perfusing fluid we used ordinary mammalian Ringer's fluid This has an osmotic pressure slightly less than that of Conger serum, but the eye and optic nerve could be left in it for several hours without any obvious harmful effect The addition of 2 p c of urea to the perfusing fluid was definitely harmful[1]

The isolated eye and optic nerve were set up in the stand shown in Fig 1 A, the eye resting in a small vulcanite chamber with the cornea touching a cover glass and kept in optical contact with it by a drop of fluid The nerve lay on two electrodes which were made of U-shaped glass tubes filled with Ringer's fluid and plugged with tufts of cotton-wool which made contact with the nerve A spiral of silver wire coated with silver chloride dipped into the other limb of the U and led off the currents to the amplifier and electrometer A third electrode was sometimes used to support the free end of the nerve and another was arranged in front of the eye for use when the retinal currents were investigated The same arrangement was used for experiments on the eye and optic nerve of the frog, except that the cover glass in front of the eye was omitted All experiments were made at room temperature, which was kept in the neighbourhood of 16° C

[1] Schulz and Krüger (*Winterstein's Handbuch*, Bd. i, p 1144) quote Delaunoy as saying that Conger serum contains 2 16 p c. of urea, though as a rule such high concentrations are found in elasmobranchs but not in teleosts Elsewhere the depression of the freezing point is given as − 75° which is much closer to that of mammalian serum (− 6°) than to that of an elasmobranch (−2 2° C.)

Illuminating apparatus

As we wished to control the area of the retina stimulated as well as the intensity of the stimulus the optical system was arranged to focus
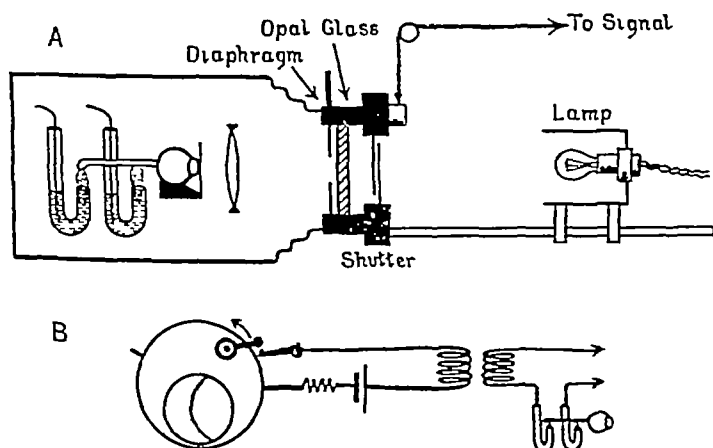

Fig. 1 A Arrangement of apparatus for focussing an image of an illuminated disc on the retina.

  B Detail of electric signal.

the image of an illuminated disc on the retina In most of the experiments the disc was of opal glass and was lit from behind by a 100 c p gas filled lamp with an opal bulb Immediately in front of the disc (i e between it and the eye) a large iris diaphragm was placed and the positions of the diaphragm lever corresponding to various areas were marked on a scale The disc was placed on the open side of the metal box which contained the preparation and all other light was excluded by black velvet curtains The front surface of the cornea was at a distance of 18 cm. from the disc and in order to focus the disc on the retina the refraction of each eye was first determined with an ophthalmoscope and the necessary lenses were then set up in a holder immediately in front of the eye

With the refraction of the front surface of the cornea annulled by the cover glass the eye removed from the body and set up in the stand always showed hypermetropia of 6-9 D According to Beer most fish examined alive are myopic but the eyeball of the eel is not at all rigid and there is probably a considerable shortening of the antero-posterior diameter when the eye is dissected out. The ophthalmoscope determinations were controlled in several cases by cutting a small window in the sclerotic at the back of the eye and immersing it in Ringer s fluid, with the cornea downwards on the stage of a microscope so that the eye would form an image of an illuminated slit placed 18 cm. below the

microscope stage Without lenses the image was always formed 1 mm. or more above the back surface of the eye and a lens of about 13 D was needed to bring it to coincide with the cut edge of the retina If the eye were emmetropic, a lens of $+5\,5$ D would be needed to focus an image at 18 cm. and the degree of hypermetropia is therefore $13 - 5\,5 = +7\,5$ D The length of the image with a $+13$ D lens in front of the eye was 1/38th of the length of the slit, the image was well defined and showed no obvious distortion.

In practice, then, if a lens of $+5\,5$ D plus the lens required to make the eye emmetropic is placed in front of the eye chamber an image of the disc, reduced to 1/38th of its diameter will be formed on the retina, and an alteration in the size of the disc will alter the area, but not the intensity of illumination of the image

The eye was arranged so that the image would fall near the centre of the retina avoiding the point of entrance of the optic nerve

The intensity of illumination of the disc was varied by placing the lamp at different distances behind it The actual illumination on the side facing the eye was found by comparing the disc with a white cardboard screen lit by a standard lamp at various distances The weakest illumination of the disc gave a match with a white surface having an illumination of 23 metre candles, the strongest illumination was equal to 830 metre candles[1] In certain experiments where more light was required a ground glass screen was substituted for the opal glass and this gave a surface approximately 16 5 times as bright When a very small image was required a pin hole with no diffusing screen took the place of the disc For very large images the eye was brought nearer the disc and the necessary lenses were interposed

Shutter and signals A large photographic shutter was placed immediately behind the disc and the speed of opening and closing was adjusted so that the largest area in use (2 cm diam) would be uncovered in 1/60 sec At faster speeds the vibration of the shutter gave rise to artefacts in the amplifier circuit Two signals were used for marking the opening of the shutter on the action current record One was a lever set in front of the slit of the recording camera and connected by a silk thread with a rotating drum which formed part of the mechanism of the shutter Tests made by reflecting the light passing through the shutter on to the slit of the recording camera showed that an interval of 1/30 sec elapsed between the appearance of the light and the movement of the signal lever, but the lag was constant to within *1/90 sec* For more exact work on latent periods an electric signal was added later The first movement of the shutter was arranged to break the current in a circuit which was inductively coupled by a few turns of

[1] The illumination of a horizontal plane on a cloudy day is about 500 metre candles, that of a plane lit by the direct rays of the sun in zenith through a clear sky is 288 000 metre candles
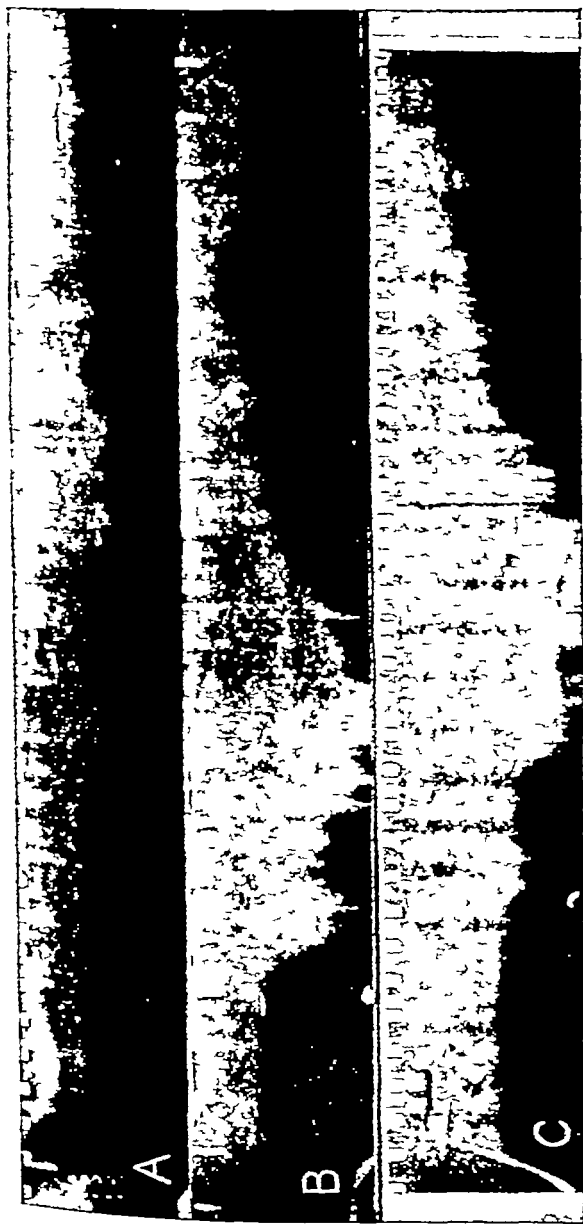
Fig 2 Film records of action currents in cat's optic nerve on exposure of the eye to light. Time marker gives 1/30 sec intervals in all film records The disc is exposed just before the strip begins Temp 16° C.

A and B Exp 28

  A Disc, diam 12.8 mm Illumination 810 metre candles.

  B Disc, diam 12.8 mm Illumination 13280 metre candles.

C Exp 21 Circulation intact. Leads from intracranial part of nerve Disc, diam 30 mm Illumination 830 metre candles

wire with the input lead of the amplifier (Fig 1 B) Breaking the circuit induced a brief change of electromotive force in the input and this appeared as a sharp spike in the electrometer record coinciding with the opening of the shutter As the movement of the shutter was not instantaneous large areas would not be fully exposed until 1/60 sec or so after the appearance of the signal Fortunately these times are small compared with the latent periods measured, which are of the order of 1/15 to 1/5 sec

Section I

Nature of optic nerve discharge

A preliminary account of this has been published already (Adrian and Eckhard(1)) As a rule the nerve is fairly quiet when the eye is in the dark, but the sudden exposure of the disc to a light of moderate intensity always produces a well-marked action current discharge Typical discharges with lights of different intensity are shown in Fig 2 The individual excursions are smaller than those usually obtained by stimulating the receptors of a peripheral nerve This would be a natural consequence of the greater number of fibres in the optic nerve, since the ratio of the recorded E M F from one fibre to that actually developed in it varies inversely with the number of fibres in the nerve trunk Apart from this difference in size, however, there is very little to distinguish the discharge in the optic nerve from that, *e g* in the frog's sciatic when the tension receptors in the gastrocnemius are stimulated by stretching the muscle

That the oscillations are due to action currents in the optic nerve fibres is shown by their disappearance when the nerve is crushed between the eyeball and the electrodes, and by their change in form when the electrodes are arranged for monophasic instead of diphasic recording Owing to the short length of nerve available the responses are never completely diphasic, but there is an obvious shortening of the first phase of the response and development of the second phase when the proximal electrode is shifted from the end of the nerve to a point nearer the distal electrode The same change in form can be obtained by leaving the preparation for several hours in Ringer's fluid after dissection and so giving the injury current time to subside The usual controls have been made to exclude artefacts

A record of the optic nerve discharge made on a rapidly moving plate is reproduced in Fig 3 with the analysis of a portion of the record below The small size of some of the excursions makes it difficult to

detect variations in the time relations of individual responses, but it does not seem likely that they differ much from one another The
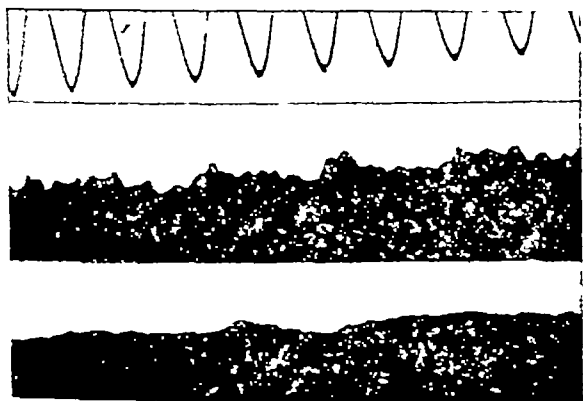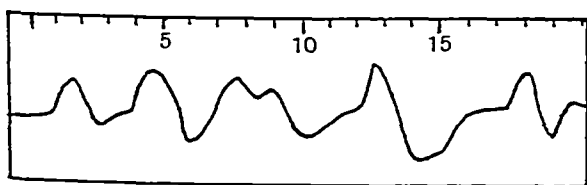

Fig 3 A.

Fig 3 B

Fig 3 A. Plate record. Time marker gives 1/100 sec. intervals.

Exp 3S. Pin hole, 5 mm. diam. Above, record just after initial outburst, below, eye in darkness.

B Analysis of portion of record in Fig 3 A. Time in 1/1000 sec. intervals

average duration of a monophasic response 0015 sec , agrees with that of the response in a peripheral sensory nerve fibre   There are certainly great differences in the diameter of the fibres which make up the optic nerve for we have found fibres of 8μ side by side with fibres of 1μ diameter This may explain the rather irregular appearance of the optic nerve records as compared with those from a peripheral nerve Although there is some variation in size as between consecutive impulses in the same record, we have found no sign of any general change in size corresponding with changes in the intensity of the stimulus and the experiments leave no doubt that there is in fact an "all-or-nothing" relation between the impulse and the stimulus as in the sensory fibres from other receptor organs

Since each fibre of the optic nerve must supply on the average an area at least 90μ diam on the retina (see p 413) it would seem to be an easy matter to restrict the activity to a single nerve fibre But in our records, even with a retinal image of 10μ diam, the impulses are evidently derived from more than one nerve fibre since considerable overlapping of action currents may occur The cause of this will be dealt with later Its practical consequence is that reducing the size of the image does not allow us to distinguish the frequency and grouping of the impulses in a single optic nerve fibre, though the frequency cannot, of course, be greater than that shown in the record

Evolution of the discharge

(a) *Steady illumination* With action currents as small as these it is impossible in continuous film records to count the total frequency of impulses per sec with any certainty when it exceeds a value of 250–300 per sec Below this limit it is only possible when the impulses are diphasic, and the photographic recording as sharp as possible

To test the accuracy of counting, control experiments were made in two sciatic nerves from the frog arranged side by side The two nerves together would contain about 6000 nerve fibres, and the action currents are about the same size as those in the eel s optic nerve Impulse discharges were produced by hanging weights on the gastrocnemii and the frequencies were counted first with the receptors of one muscle excited, then with the other, and finally with both together If the conditions of excitation are kept constant the frequency of the impulses when both sets of receptors are in action should be the sum of the frequencies with each set acting separately Provided that the frequency did not exceed 300 per sec the counts agreed fairly well with this, though there is a tendency to underestimate the number when the frequency is as high as 200 and the error becomes greater and greater at higher frequencies owing to the overlapping of the different impulses.

The maximum frequency of the discharge can be kept below 300 per sec by controlling either the size of the retinal image or the intensity of the light and it is then possible to follow the evolution of the discharge when the shutter is opened suddenly and remains open for several seconds Examples from two experiments are given in Figs 4 and 5 They show a discharge which begins after an appreciable latent period, rises rapidly to a maximum and then declines more slowly When the light is turned off there is a sudden increase in frequency and then a drop to zero Both the decline in frequency during stimulation and the renewed outburst on darkening were unexpected results but we have found them in every preparation (more than 30 in all)

Consideration of the latent period of the discharge must be deferred to the next section since it is related to the latent period of the retinal
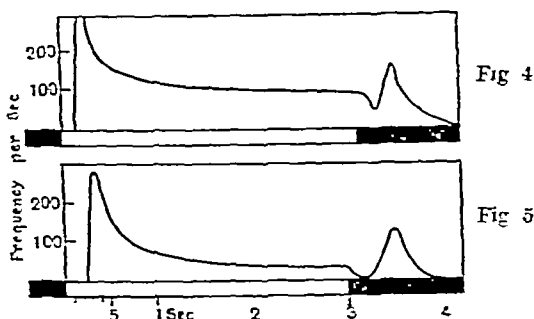

Fig 4. Frequency of impulses per sec during and after illumination. Duration of lighting shown below record. Exp. 16 Disc, diam. 36 mm. Illumination 830 metre candles

Fig 5. Ditto. Exp. 13 Disc, diam. 12 8 mm. Illumination 830 metre candles

current As regards the decline in the frequency of the discharge although an uncritical examination would tell us that an object appears equally bright as long as we look at it there is no doubt that the brightness does in fact decline considerably from its initial value If we prevent the eye from moving and look at the dim outlines of an object in a dark room in 20 sec or less the object disappears and the field becomes uniformly blurred and even in a bright light outlines soon become hazy if we maintain complete fixation (Hartridge(4), see also Dav(5)) It is hard to say whether there is a difference in the rate of decline of the impulse frequency curve with lights of different intensity as we are limited to frequencies below 300 per sec In some preparations with small areas and faint lights the discharge fails altogether after one or two seconds whereas with bright lights and larger areas the frequency declines very slowly after the rapid initial fall (as in Fig 4)

The form of the frequency curve for a given stimulus varies considerably from one preparation to another as does the sensitiveness of the eye to light[1] We have not found that repeated short exposures (less than 10 sec) to light of moderate intensity produces any change in the discharge provided that the eye remains in the dark from 10–20 sec

[1] The eye was protected from the light during the dissection and was then set up in the dark preparation box, too wit to per use being about half an hour Further use in the dark does n t seem to move up or reduce sense How ed our preparations have failed to give any response to any eye one of the disc was had a diameter of 3 2 mm and an illumina t on of more than 2 metre candles.

between each exposure With very strong lights (16,000 м c illumina-
tion of disc) longer intervals of rest have to be allowed

As the preparation is isolated from the body it seemed possible that
the decline in the discharge might be due partly to the abnormal con-
dition of the eye It is known that in a freshly excised eyeball the
retinal currents produced by exposure to light do not differ considerably
from those observed when the eye is left *in situ* with the circulation
intact, and Westerlund(6) has shown that a frog's eye must be kept
for 2 hours in nitrogen before the retinal currents disappear completely
But the excised eye does become progressively less responsive as judged
by the retinal currents and it is quite possible that the processes con-
cerned in the optic nerve discharge might be more easily upset than
those concerned in the production of the retinal currents To test this
point we made two experiments on the eel's eye with intact circulation,
the leads being taken from the intracranial portion of the optic nerve,
but both showed a decline in the frequency of the discharge with steady
illumination, and the rate of decline was no less rapid than in the average
excised preparation A record from one of these is given in Fig 2

Thus the decline in the frequency of the discharge must be accepted
as a normal occurrence in the eel's eye, and although the experiments
are less accurate we are satisfied that it occurs in the frog's eye as well
This brings the optic nerve discharge into line with that from peripheral
receptor organs, for in every one that has been examined there is a
decline in frequency with constant stimulation In the peripheral re-
ceptors this decline in frequency has been related to the "adaptation"
which occurs when a nerve fibre is stimulated with a constant current
In the nerve fibre the adaptation to the stimulus is so rapid that only
one impulse is produced It is slower in the skin receptors and very
slow indeed in the receptors in muscle which are concerned with the
long continued postural reflexes Whether the decline in the discharge
from the eel's eye is due to an adaptation of this kind (i e a process
which is the direct consequence of the stimulus) or to fatigue (the
consequence of the activity of the organ) is a question which must be
left open for the present, but the functional importance of the decline
is clear It means that a changing visual field will produce a greater
sensory effect than a stationary field, i e that a movement will be more
readily perceived than a steady pattern of light and shade

This is easily seen by placing in front of the preparation a large disc
of oiled paper (15 cm diam) lit from behind and causing a shadow to
move across the disc When the shadow moves, the retinal points which

pass from darkness to light will be strongly excited and the impulse discharge during the movement is far greater than it is when the whole field has been evenly illuminated for a few seconds (Fig 6)

Fig 6 Movement of shadow across the visual field. Large illuminated screen in front of the eye A sector of the screen is shaded and in the middle of the record this is moved across the field.

It is generally agreed that in animals whose visual sense is not strongly developed the eye is mainly an organ for the perception of movement but our results do not exclude the possibility of some kind of pattern perception as well Movements of the eye relative to a stationary visual field would have the same effect as a movement of the field In man it is known that there are constant small oscillations of the eyeball which occur even during the most careful fixation If these occur in the eel a steady field consisting of a bright disc on a dark background would produce a circle of strongly excited retinal points since these points would be continually passing from light to shadow and *vice versa* Whether such movements do occur in the eel is extremely doubtful and in man we do not know whether they have any real function, though it seems likely that they do serve a useful purpose in maintaining the retinal excitation (cf. Dodge(7))

There is another point which must not be lost sight of, and that is that the curves in Figs 4 and 5 show a rapid decline in the frequency of the impulses occurring in a group of several nerve fibres, but we cannot assert that the rate of decline is the same in every fibre It is possible that the evolution of the discharge arising in the cones may have different time relations from that of the rods Our records do not show any distinct evidence of two different kinds of response and as the eel's retina has no fovea it has been impossible to test the point directly The only indication of a dual mechanism is the occasional occurrence of a preliminary rise in frequency followed by a decline

before the maximum frequency is reached   An indication of this appears
in Fig 2   It is often absent even with strong lights

Stimuli of short duration   the "off effect"   A typical response to
a very brief illumination of the disc is shown in Fig 7   The discharge
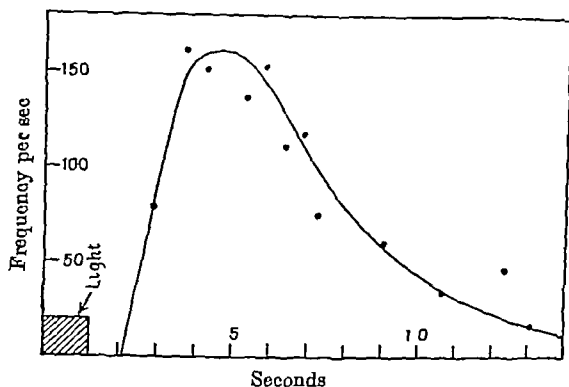

Fig 7   Response to flash of light lasting 125 sec (approx.)   Exp 28   Disc, 36 mm. diam
Illumination 830 metre candles

begins some time after the light is shut off and lasts for about 1 sec
A discharge of much the same form dwindling to nothing after a *second*
is often obtained by the continuous exposure of a small and feebly
illuminated area   But with stronger lights and larger areas there is
always a definite "off effect" provided that the stimulus has lasted for
a second or more   The off effect has a latent period comparable to that
of the initial discharge, it lasts for about 1/5 to 1/2 sec , dying away
gradually   The maximum frequency shows a decided increase as the
duration of the preceding stimulus is prolonged and it may be fully
equal to that of the initial discharge when the illumination has lasted
10 sec or more   Typical "on" and "off" outbursts from the same
record are given in Fig 8 A   Fig 8 B shows the same thing in the frog's
optic nerve and here the "off" outburst is longer than the "on"   The
preparation which gave this record was an exceptionally good one—
as a rule the base line has been unsteady in records from the frog

For the present the chief interest of the "off effect" lies in its relation
to the retinal currents, which will be discussed in Section II, and in its
functional interpretation   There is not much to be said about the latter
Since the discharge from the retina may decline almost to nothing under
a steady illumination it is clearly to the advantage of the organism to
have an eye which will signal the change from light to darkness, as well
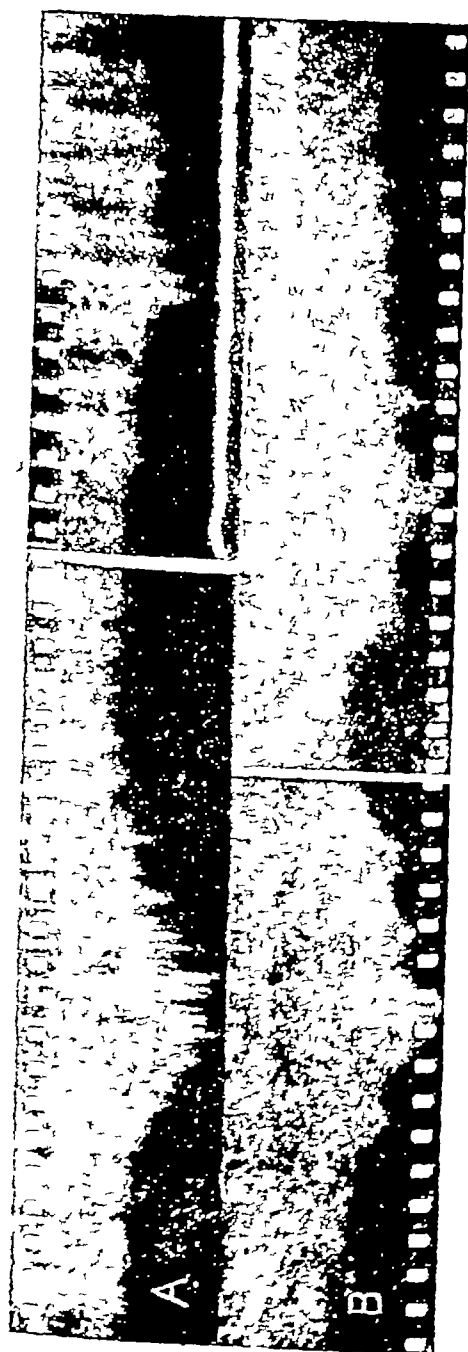
Fig 8 "On" and "off" outbursts. Portions of film from the sumo records

A Exp 10 Eel Disc, 12 8 mm diam Illumination 208 metre candles.
B Exp 20 Frog Disc, 26 mm diam Illumination 208 metre candles

as that from dark to light, but it seems difficult to correlate so great a discharge with any of the visual sensations which we experience when a light is turned off   However, it may be a mistake to regard the discharge as one of considerable intensity, for there is no reason to suppose that the impulses which make up the "off effect" are travelling in the same nerve fibres as those which make up the primary discharge   They may be derived from the surrounding parts of the retina and may represent a low frequency discharge in a large number of nerve fibres whereas the primary effect is a high frequency discharge in a few fibres

Frequency of discharge in relation to intensity and area   When the illumination of the disc is increased without any change of area, the impulse discharge in the optic nerve shows three changes   The latent period is reduced, the maximum frequency of the discharge is increased, and the maximum frequency is reached at a shorter interval after the beginning of the discharge   These changes can be seen in Fig 9   Fig 10 shows the effect of intensity on the latent period, and Fig 11 gives six curves showing its effect on the maximum frequency of the discharge   The number of points is not great enough to allow the equation of the curve to be stated, but the frequency is evidently some exponential function of the intensity   Such a relation is not surprising Curves of much the same form have been obtained from other receptors, and the form is in general agreement with what is known of the relation between the stimulus and the brightness of the sensation in man

The effect of increasing the size of the retinal image without altering the intensity of illumination was quite unexpected   Thinking of the retina as composed of a number of small areas each connected with a single nerve fibre, we were prepared to find that the only change would be in the frequency of the discharge and that this would be directly proportional to the area of the disc which is focussed on the retina What we found was a reduction in the latent period of the discharge, a quicker rise to the maximum and an increase in frequency which was not nearly proportional to the area stimulated

Curves showing the evolution of the discharge for different areas are given in Fig 12   Fig 13 gives the effect of area on the latent period, and Fig 14 shows its effect on the maximum frequency of the discharge   The effect of increasing the area is so much like that of increasing the intensity of the light that we supposed at first that the focussing of the image was at fault and that an increase in the area of the disc would not produce a corresponding increase in the area of the retinal image, but would increase its intensity of illumination
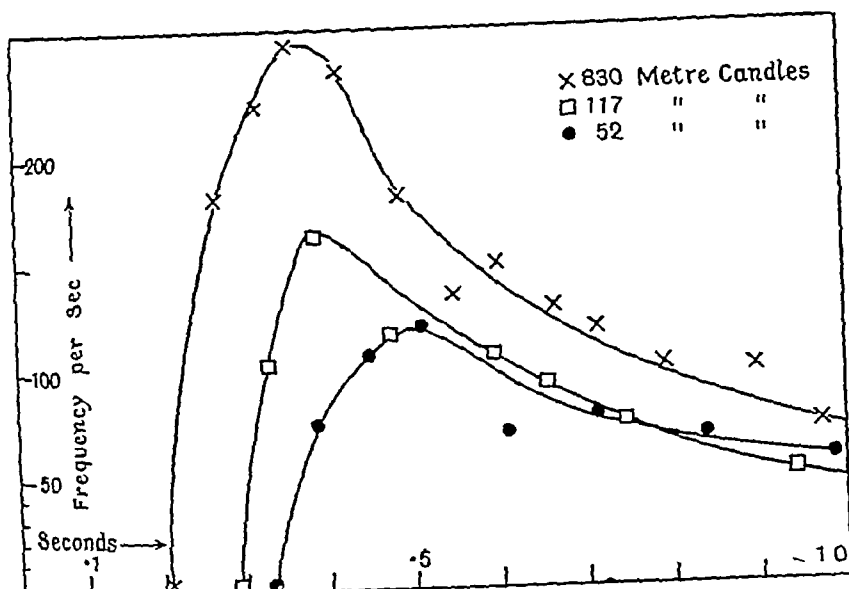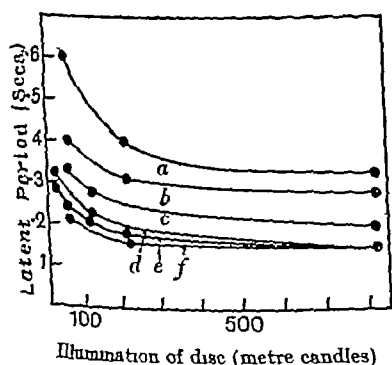
Fig 9

Illumination of disc (metre candles)

Fig 10
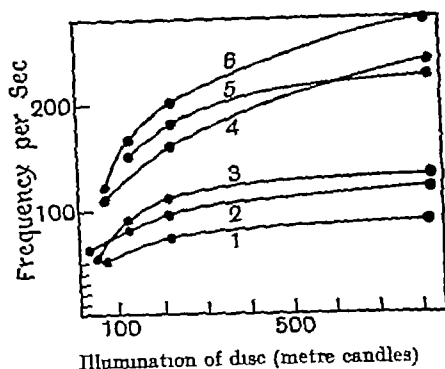

Illumination of disc (metre candles)

Fig 11

Fig. 9 Evolution of discharge with different intensities of illumination. Exp 28 Disc, 36 mm. diam. Illumination 830, 117 and 52 metre candles.

Fig 10 Change in latent period with intensity of illumination.

a	Exp 17 Disc, 25·5 mm. diam.	d	Exp 16 Disc, 3·2 mm. diam.
b	Exp. 33 Disc, 36 mm. diam.	e	Exp 29 Disc, 25·5 mm. diam.
c	Exp 28 Disc, 25·5 mm. diam.	f	Exp 27 Disc, 25·5 mm. diam.

Fig. 11. Maximal frequency of discharge with different intensities.

1	Exp 17 Disc, 6·4 mm. diam.	4.	Exp 38 Disc, 36 mm. diam.
2	Exp 23 Disc, 3·2 mm. diam.	5	Exp 20 Disc, 36 mm. diam.
3	Exp 16 Disc, 3·2 mm. diam.	6	Exp 28 Disc, 36 mm. diam.

But repeated tests have shown that the optical system does produce a reasonably sharp image on the retina, and we have tried the experiment of placing a series of lenses in front of the eye ranging from + 13 D (which should give a sharp focus) down to − 8 5 D without finding one which altered the form of the relation between area and frequency There is no doubt a large error in the counting of the different frequencies since it is always difficult to know how many impulses go to make up the large spikes which appear in the records of high frequency discharges, but it is scarcely conceivable that the error is large enough to make the frequency appear to be only doubled when it has really increased fifty-fold When the responses are diphasic there is always a possibility of interference between the opposite phases of two impulses which occur very close together, but this can be eliminated by using monophasic leads It is then more difficult to count the individual excursions, but an approximation to the total frequency can be obtained by determining the initial movement of the electrometer away from the base line This method is only applicable to high frequencies, but it shows the same kind of relation between frequency and area as that obtained by direct counting

Since an increase in area with the intensity constant has the same effect as an increase in intensity with area constant, it seemed possible that, under the conditions of our experiments, the character of the discharge is really determined by the total quantity of light which falls on the retina in unit time, without regard to the distribution of the light This possibility can be tested by making comparisons of the discharges produced by keeping the quantity of light in unit time constant and varying the area and the intensity The characteristic which can be measured with the greatest accuracy is the latent period of the discharge, and Fig 15 A and B give two curves for the eel and the frog showing the relation between the latent period and the quantity of light in unit time The different points refer to retinal images of widely different area, but all of them fall on the same curve when the latent period is related to the quantity of light (area × intensity) This relation has been investigated in eight experiments, with discs ranging from 8 to 512 mm ^{2} (corresponding to retinal images from 08 to 67 mm diam), in a few the results have been less consistent but none of them have shown any clear divergence from the relation as between large and small areas We have only two experiments in which it is possible to make a direct count of the frequencies with area and intensity both varying The data for one of these is shown in Fig 16 and
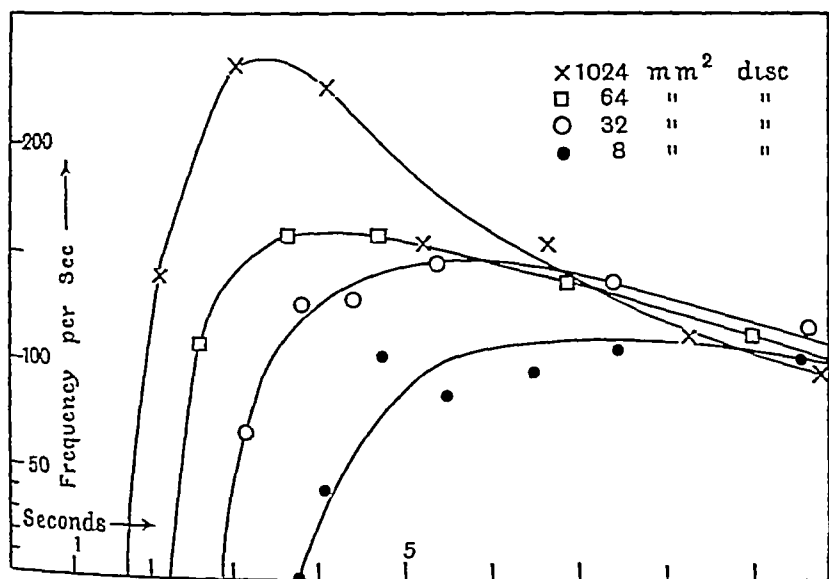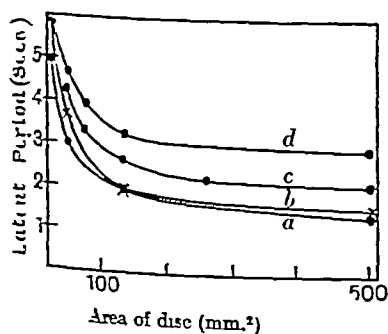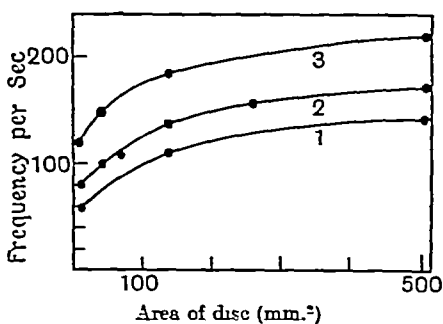
Fig 12

Fig 13

Fig 14.

Fig. 12. Evolution of discharge with discs of different area. Exp 28 (as in Fig 9) Illumination of disc 830 metre candles.

Fig. 13. Change in latent period with area of disc.

a Exp 27 Illumination 830 metre candles.
b Exp. 29 Illumination 208 metre candles.
c. Exp 28. Illumination 830 metre candles.
d Exp 33 Illumination 830 metre candles.

Fig. 14 Maximal frequency of discharge with different areas.

1. Exp. 18 Illumination 208 metre candles.
2. Exp 28 Illumination 830 metre candles.
3. Exp 22. Illumination 23 metre candles.

N B A disc of area 8 mm.² gives a retinal image ·084 mm. diam.
"   32   "   168   "
"   128   "   "   338   "
"   512   "   "   67   "

it will be seen that here too the maximum frequencies obtained for the same quantities of light are the same within the rather wide limits of variation found when similar exposures are repeated

It is obvious that these facts do not agree with the simple conception of the retina as a mosaic of sensitive points each connected with its own nerve fibre and each acting independently of the rest   The shortening of the latent period when the size of the retinal image is increased can only be explained on the assumption of some kind of interaction between the different stimulated points   The dependence of the discharge on the quantity of light without regard to its distribution seems to involve a process of the same kind

We can gain some idea of the extent of this interaction by finding within what limits an increase in area copies the effects of an increase in intensity   In our experiments on the effect of area on latent period the largest areas correspond to a retinal image of 1 mm diam   An increase in the diameter of the image from 6 mm to 1 mm produces a slight decrease in the latent period, but the form of the curve suggests that with retinal images of diameter above 1 mm the latent period would no longer alter as the area is increased   To test this point two experiments were made with retinal images up to 2 mm diam by moving the eye nearer to the illuminated disc and interposing the necessary lenses to focus the image   In both of these an increase in the image from 1 mm to 2 mm diam produced a decrease in the latent period which was so slight that it was well within the range of experimental error   On the other hand much shorter latent periods were obtained by increasing the intensity of the light

Thus the change of latent period with area is either absent or very slight when the diameter of the retinal image exceeds 1 mm   With images between 6 mm and 1 mm diam the effect is present but it is very much greater when the diameter is reduced below 3 mm   Whether the frequency of the discharge becomes more nearly proportional to the size of the image when this exceeds 1 mm diam is a question which must be left open till we have further data, but the lack of proportionality is certainly greatest with very small images

Having obtained this unexpected result we found that we ought in fact to have anticipated it   It has been known for many years that in the human eye the threshold intensity of illumination for small areas varies with the size of the area   The effect begins to appear when the diameter of the retinal image is reduced to about 5 mm [1], and as a

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[1] For foveal vision Charpentier found that this relation was only obeyed when the
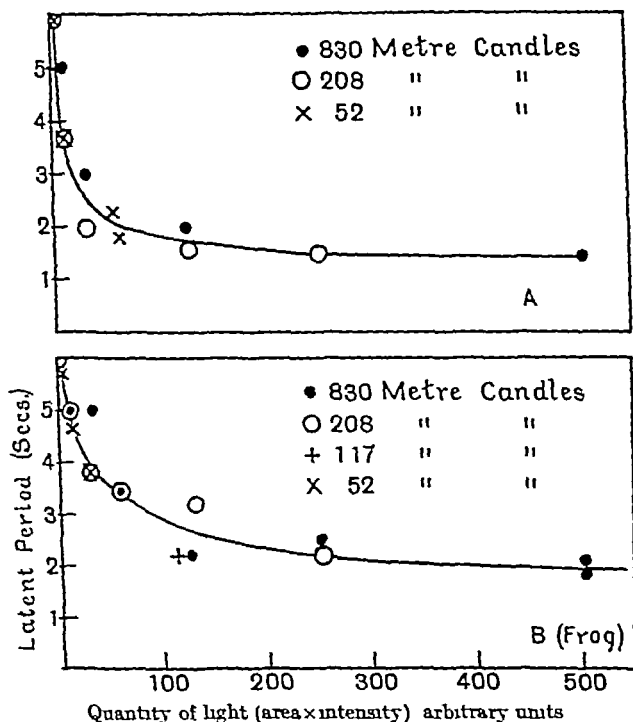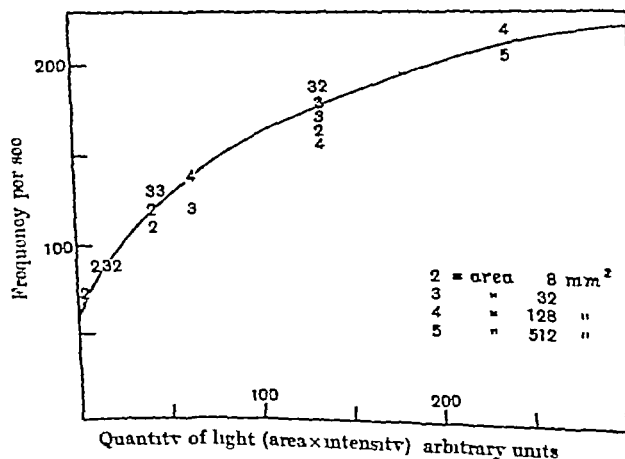
Fig. 15. Relation between latent period and quantity of light (area × intensity), expressed in arbitrary units.

A. Exp. 27 (Eel).   B   Exp. 30 (Frog)

Fig 16   Relation between maximal frequency of discharge and quantity of light. Exp 23 (Eel).

rough approximation it is found that the product of intensity and area must be kept constant if the image is to be just visible   The work of Abney(8) and the more recent investigations of Prentice Reeves(9) and of Pieron(10) have shown that the relation between intensity and area is really more complex, since the quantity of light increases gradually as the area is enlarged   According to Abney the effectiveness of the stimulus seems to depend more on the linear dimensions of the retinal image than on its area and if the image is oblong instead of circular, the smallest diameter is the determining factor

There can be little doubt that the effects we have observed are closely related to these phenomena of human vision   It is known that the visual reaction time in man depends on the intensity of the light when the area is constant, and since the effectiveness of a given intensity increases with the area we should naturally expect a change in latent period when the intensity is constant and the area is changed   Our own results on the eel and the frog are not accurate enough to show whether the quantity of light required to give a certain latent period does or does not increase slowly with area, as does the quantity required for minimal sensation in man, but it is clear that an increase must take place when the retinal image is greater than 1 mm. diam

It is remarkable that the range of areas over which the effects are observed are so closely alike   In the eye of the conger and of the frog we find that an increase in the retinal image above 1 mm diam ceases to make an appreciable difference to the latent period, in the human eye Abney's figures show that the size of the image ceases to affect the threshold intensity when it is greater than about 8 mm though other observers give a smaller range

In a structure as complex as the vertebrate retina the latent period is probably the sum of a number of distinct processes, and it seems essential to know something more of these before we can attempt to explain the change of latent period with area   Fortunately we can learn something definite from the relation between the retinal currents and the optic nerve discharge, and we shall therefore discuss this before dealing with the effects of area on the frequency of the discharge

retinal image did not exceed the diameter of the fovea (17 mm.) Abney found that for vision with the centre of the retina the threshold intensity was still affected by the size of the image up to about 8 mm. (3° visual angle)

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SECTION II

The retinal currents

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The retinal currents appear as changes of potential which may reach several millivolts between the front surface of the cornea and the back of the eyeball or any structure connected with it (*e g* the optic nerve, the brain, or indeed any part of the body) when the eye is exposed to light They are known to be changes occurring in the retina for they are still obtained after the front of the eyeball is removed and Bovie, Chaffee and Hampson have recorded very large effects by leading off directly from the retinal surface It is uncertain however, to what part of the retinal apparatus they belong Bovie, Chaffee and Hampson(11) suggest that they are the summed effects of action currents in the nerve fibres and processes, whilst Gotch(12) and others have regarded their slow time relations as indicating an essential difference from the response of nerve

The potential changes which occur when a light is turned on are complex and probably represent the sum of several independent processes Einthoven(13) and Piper(14) regard them as due to three processes and Bovie, Chaffee and Hampson find an even greater complexity But the general course of the potential change is fairly clear and we may take Einthoven's account as giving a convenient method of describing it The effects produced by Einthoven's three processes A B and C are shown in Fig 17, together with the actual change of potential resulting from them.
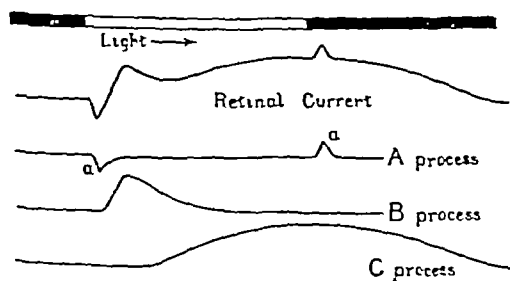

Fig. 17 General form of retinal current and Einthoven's analysis into three processes A, B and C

When the light is turned on there is first of all a negative deflection (*a*), the current passing through the external circuit from the back of the eye to the cornea This is followed by a positive deflection (due

to B) which decreases under steady illumination, but may increase again owing to the development of the slower C process   When the light is turned off there is a rapid positive deflection (a') and finally a slow return to the resting condition   Einthoven regards the initial negative deflection a and the rapid positive deflection on darkening a' as due to the same process   The positive deflection which succeeds a is due to the B process and is best seen with feeble illumination   Finally the C process is a very much slower change which may remain for many seconds after the light is turned off
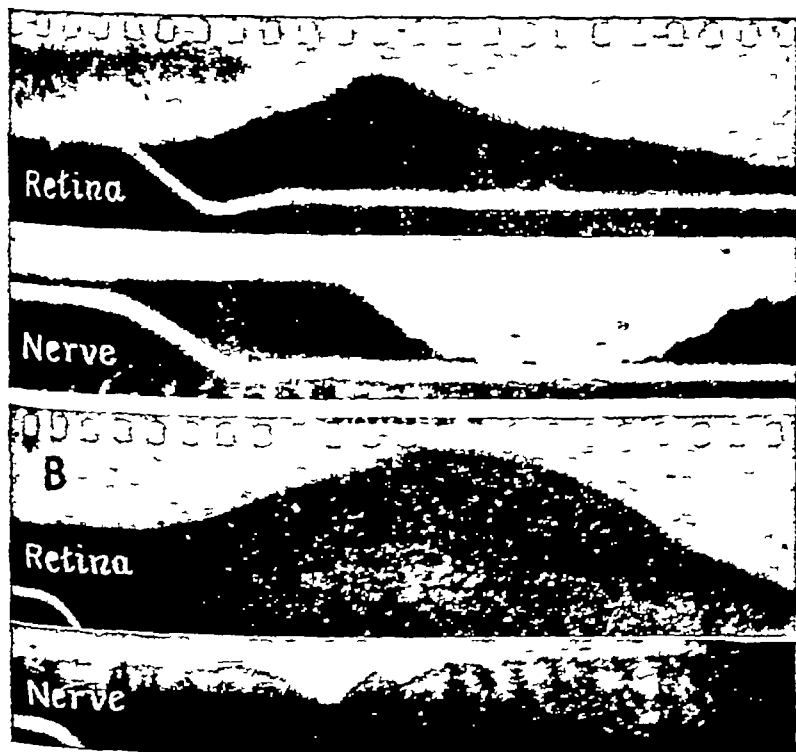
In a valuable paper by Day(5) on the retinal currents in fish there is a discussion of the relation of these different processes (or deflections) to the production of visual sensation   It is generally agreed that the C process rises and declines far too slowly for it to be concerned in the discharge of visual impulses, of the other two, A and B, such evidence as there is points to the A process as being the more important   This is strongly supported by the present experiments   The records already described show that the rapid positive deflection a' when the light is turned off has a counterpart in the "off effect" in the optic nerve discharge, and from what follows it will be clear that the initial negative deflection a is closely associated with the initial outburst of impulses in the nerve

In the experiments we have made on the retinal currents we have been handicapped to some extent by the fact that our amplifier is not strictly aperiodic   A change of potential which rises suddenly and remains at a constant value will appear in the record as one rising suddenly and then declining to zero   The rate of decline with the condensers at present in use is such that the recorded potential falls to half its initial value in about half a second.   This makes little difference to the initial deflection a, but the subsequent evolution of the curve would be considerably distorted and to study it in detail would need an amplifier with conductive coupling such as that used by Bovie, Chaffee and Hampson   We have not attempted to modify the amplifier in this way for our experiments have been concerned almost entirely with the relation between the latent period of the "a ' effect and that of the optic nerve discharge.

The "a" effect   The eye and optic nerve preparation was arranged with the usual electrodes on the optic nerve and with two more leading from the front and back of the eyeball   Either pair could be connected with the amplifier by a double pole switch, and records were made alternately from the nerve and from the eyeball

Two pairs of records are given in Fig 18   They show the a effect as a smooth deflection occurring after a latent period which is considerably shorter than that of the corresponding optic nerve discharge, in Fig 18 A the nerve discharge begins very slightly before the a effect has reached

its maximum in Fig 18 B the nerve discharge begins long before the maximum *a* effect   But in any one preparation it is noteworthy that

to B) which decreases under steady illumination, but may increase again owing to the development of the slower C process When the light is turned off there is a rapid positive deflection (a') and finally a slow return to the resting condition Einthoven regards the initial negative deflection a and the rapid positive deflection on darkening a' as due to the same process The positive deflection which succeeds a is due to the B process and is best seen with feeble illumination Finally the C process is a very much slower change which may remain for many seconds after the light is turned off

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the frog, though we have found intervals as long as 2 sec and as short as 05 sec We have not found that it changes appreciably during the course of an experiment lasting an hour or more, but we have made no systematic investigations of the effects of fatigue, temperature, etc

The beginning of the nerve discharge seems to be definitely related to the beginning of the retinal effect, it does not correspond to the development of a particular potential in the retina or a particular rate of change of potential Since there are two synaptic layers intervening between the retinal elements and the fibres of the optic nerve there is ample reason to expect a delay in conduction, but it is of great interest to find that this delay is constant and that the changes of latent period are all due to the process which expresses itself as the retinal effect It is, of course, impossible to say what part of the retinal apparatus produces the *a* deflection, but the relatively long retinal-nerve interval suggests that the seat of the retinal effect is separated from the optic nerve fibres by a relatively slow conduction path It seems possible, therefore, that the seat of the retinal effect is in the rod and cone layer and not in the synaptic networks and that the constant retinal-nerve interval is due to the conduction through the latter If this is so the variation in latent period with intensity and area must be due to variations in the rate of development of the preliminary process which excites the rods and cones (*i e* the photochemical change) and not to summation effects in the synaptic layers

There are two lines of evidence which strengthen this view Hecht's analysis of the reactions of Mya and Ciona(15) to light shows that the changes in the latency of response in this case can all be related to the preliminary photochemical and catalytic changes which lead up to the stimulation of the nervous structures The other line of evidence is less conclusive but still worth mentioning In his work on the relation of area to threshold intensity Abney found that the effect of increasing area was practically the same for direct vision as for vision at an angle of 18° from the optic axis The nervous arrangements in the fovea and in the periphery of the retina are so different that it is hard to believe that interaction in the synaptic layers could give the same quantitative results in the two situations On the other hand, we know so little of the photochemical mechanism, that it is at least arguable that it does not differ much in different parts of the retina

The B effect How far the later developments of the retinal current are related to the optic nerve discharge we are not prepared to say In the eel the negative *a* deflection is succeeded after about 2 sec by the positive

optic nerve impulses have been appreciably distorted by interference from the retinal currents

The most important point, however, is the constant interval between the beginning of the *a* effect and the beginning of the optic nerve discharge   This is shown with greater certainty in Fig 19, which gives the
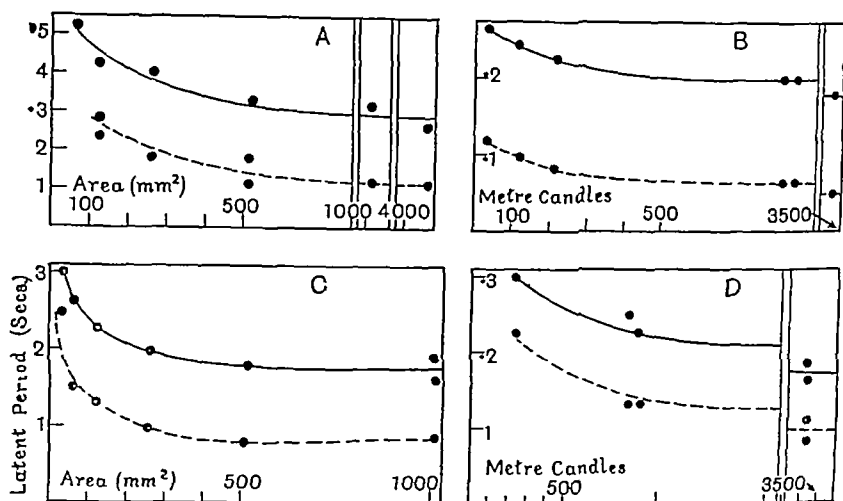

Fig 19   Latent periods of retinal reponse (*a*) and nerve discharge, showing constant "retinal nerve" interval.

A. Exp 37   Frog   Illumination of disc, 32 metre candles
B   Exp 37   Frog   Diam. of disc, 72 mm. (4096 mm ²)
C   Exp 31   Eel.   Illumination of disc, 860 metre candles
D   Exp 31   Eel   Diam. of disc, 12 8 mm.

latent periods of the two in various experiments with changing intensity and area   With the weakest lights it is often impossible to measure the latent period of the retinal effect, as it is very small and develops very gradually, but there is no sign of any constant change in the retinal-nerve interval as long as it can be measured   This statement should of course be qualified by a reference to the limits of accuracy of these measurements, which are not very great   Even in our best experiments we must admit a variation of 20 p c   either way in the retinal-nerve interval, but the fact remains that the interval shows no sign of a definite increase or decrease when the latent period of the retinal effect is doubled or trebled   Thus the nervous discharge appears at a fixed time after the beginning of the retinal effect whatever the latent period of the latter may be   The retinal-nerve interval varies from one preparation to another   its average value is 1 sec   both in the eel and

the frog, though we have found intervals as long as 2 sec and as short as ·05 sec. We have not found that it changes appreciably during the course of an experiment lasting an hour or more, but we have made no systematic investigations of the effects of fatigue, temperature, etc.

The beginning of the nerve discharge seems to be definitely related to the beginning of the retinal effect, it does not correspond to the development of a particular potential in the retina or a particular rate of change of potential. Since there are two synaptic layers intervening between the retinal elements and the fibres of the optic nerve there is ample reason to expect a delay in conduction, but it is of great interest to find that this delay is constant and that the changes of latent period are all due to the process which expresses itself as the retinal effect. It is, of course, impossible to say what part of the retinal apparatus produces the *a* deflection. but the relatively long retinal-nerve interval suggests that the seat of the retinal effect is separated from the optic nerve fibres by a relatively slow conduction path. It seems possible, therefore, that the seat of the retinal effect is in the rod and cone layer and not in the synaptic networks and that the constant retinal-nerve interval is due to the conduction through the latter. If this is so the variation in latent period with intensity and area must be due to variations in the rate of development of the preliminary process which excites the rods and cones (i.e. the photo-chemical change) and not to summation effects in the synaptic layers.

B deflection, but it is impossible to specify the moment at which the a process ends or that at which the B process begins, for the curve shows no discontinuity but passes smoothly from the negative to the positive deflection   In many experiments the maximum discharge in the nerve is reached well before the retinal effect changes from negative to positive In the frog the duration of the a deflection is often shorter than in the eel, the average being 13 sec

The work of Bovie, Chaffee and Hampson suggests that the magnitude of the B effect is closely related to the activity of the nervous elements of the retina, but the difficulty of separating the opposing changes of potential of the a and the B effects has prevented us from making any useful comparison of the B effect and the optic nerve discharge

The "off effect" (a')   When the light is turned off after an illumination lasting a second or more the retinal current shows the positive deflection a' which Einthoven regards as due to the same process as that responsible for the initial negative deflection a The a' effect is greater the longer the eye has been exposed to light and in general the conditions which favour its development are the same as those favouring the development of the off effect in the optic nerve discharge

By recording the retinal and optic nerve currents with exposures of several seconds' duration it is possible to measure the latent periods of the a and a' deflections and of the "on" and "off" outbursts in the optic nerve   This gives us two retinal-nerve intervals   We are not prepared to say whether the retinal-nerve interval for the "off" effect is identical with that for the "on "   They are certainly of the same order

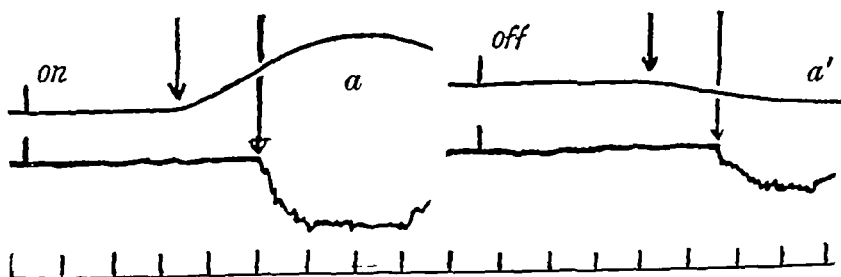

Fig 20   Tracings from records showing "on" and "off" effects from retina and nerve and "retinal nerve" intervals. Time marker gives 1/30 sec   Exp 40   Disc, 25 5 mm diam., 830 metre candles.

but in the only full scale experiment we have made, the average value for the "off" interval is slightly shorter than for the "on "   This may

be due to the much smaller size and slower development of the a' deflection compared with the a, for we measure the beginning of the effect by the first visible deflection of the base line, and we may therefore overestimate the latent period of the a' effect as compared with that of the a The difficulty in fixing the beginning may be seen from the tracings in Fig 20 The full data from this experiment are given in Table I They leave little doubt that the relation between the a' effect and the concluding outburst of impulses is of the same nature as that between the a effect and the initial outburst The difference between the two intervals, though it may be significant, is scarcely outside the range of experimental error The "off" interval is the more constant of the two, the "on" interval showing an unusual increase with the weaker lights

TABLE I.

Exp 40 16° C. Eel. Area of disc, 512 mm.²

Latent periods measured in units of 1/30 sec. to nearest 1/60 sec.

Illumination of disc (metre candles)	Light On Latent period Retina (a)	Nerve	Retinal nerve interval	Light Off Latent period Retina (a)	Nerve	Retinal nerve interval
3410	1 5	3 5	2 0	2 0	3 5	1 5
1930	2·0	4·0	2 0	1 5	3 5	2 0
860	2 5	5 0	2 5	2 5	4 5	2 0
830	3·0	5·0	2 0	3·0	5·0	2 0
203	2 5	6 0	3 5	3 5	5 5	2 0
117	4·0	7 5	3 5	4 0	6 5	2 5

Average interval 2 6 = ·087 sec.   Average interval 2 0 = ·067 sec.

Intensity of retinal effect and frequency of nerve discharge

The magnitude of the a deflection depends on the area and intensity of the stimulus and the relations have the same general form as those for the frequency of impulses in the optic nerve The main difference lies in the fact that the frequency of impulse discharge seems to have a definite lower limit (about 50 per sec), whereas the retinal response does not But this means very little for the retinal response in the eel's eye is less easy to detect than the nerve discharge and a stimulus which is just strong enough to produce a discharge of impulses is rarely strong enough to give a perceptible a deflection. We have no data which allow a detailed comparison of the impulse frequency and the magnitude of the a deflection in the same preparation, but we hope to investigate this at a later stage when the nature of the a effect is clearer and when we have modified our apparatus to enable it to record impulse frequencies over 300 per sec

Comparison with Fröhlich's results

In all our experiments, apart from such obvious artefacts as those caused, *e g* by mechanical vibration, etc , the potential changes obtained by leading off from the eyeball are smooth curves and show none of the discontinuities which appear in the records of the optic nerve discharge This may seem surprising in view of the fact that fibres of the optic nerve run in the retina for the first part of their course and might therefore be expected to contribute something to the retinal current However, the impulses in the intraorbital part of the fibres would be very unlikely to produce changes of potential in the recording circuit at all comparable with the changes which can be detected in the optic nerve proper Since the electrodes for the retinal effect are placed on the front and back of the eyeball, the intraorbital nerve fibres are running for the most part at right angles to the line joining the electrodes and their action currents will therefore have little effect on the recording circuit In addition to this the presence of a large bulk of indifferent tissue would short circuit any nerve action currents which might have a component parallel to the line joining the electrodes

A large number of monophasic action currents of slow time relations would certainly give rise to a smooth curve, but the character of the retinal effect seems to be more in accord with the view that it is determined by the photochemical changes which precede and cause the nervous excitation On the other hand, in string galvanometer records from the eye of *Octopus* and of *Eledone moschata* Fröhlich[2] finds definite oscillations with a frequency ranging from 30 to 90 per sec , the frequency depending on the intensity and the wave length of the light These oscillations were superimposed on a steady deflection, they were not always present, and they were not found in records with both leads on the optic nerve, but only in those with the retina included in the circuit The structure of the cephalopod retina differs considerably from the vertebrate type, and the most likely explanation seems to be that the response in the cephalopod eye is made up of action currents in the nervous elements superimposed on the retinal effect[1] Einthoven and Jolly[13] have noted the occasional appearance of slower fluctuations of the retinal current (2–3 per sec), but these too have been absent in our experiments

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[1] Since this was written we have found that the illumination of very large areas of the retina may give rise to an optic nerve discharge with rhythmical changes in frequency closely resembling some of Fröhlich's records These results will be published shortly

Section III

The action of light on the retina

It is clear that a great deal of work remains to be done before we can be sure of the precise significance of the retinal response as a link between the stimulus and the flow of impulses in the optic nerve In particular we hope that a detailed analysis of the latent period may show us whether the *a* response can be related to the preliminary changes which precede the nervous excitation in Hecht s scheme for Mya and Ciona But we can at least be sure that the processes which account for the effects of area and intensity on the latency of the optic nerve discharge are all processes intervening between the incidence of light on the retina and the development of the *a* response There is a considerable delay between the beginning of the *a* response and the beginning of the optic nerve discharge, but it is a constant quantity unaffected by the nature of the stimulus

The results of Section I have left us with the following facts to explain

(1) However much we restrict the size of the retinal image we seem unable to confine the impulse discharge to a single fibre of the optic nerve

(2) If we increase the area of the retinal image within limits, the frequency of the impulse discharge is not increased in direct proportion to the area but much less rapidly, and the latent period of the discharge is reduced

(3) The effects of an increase of area with constant intensity run parallel with the effects of an increase of intensity with area constant, and the nature of the discharge is determined within limits by the quantity of light without regard to its distribution

If we leave out of account all considerations based on the power of visual discrimination, etc , in the human eye, these results are most simply explained by assuming that the incidence of light on restricted areas of the retina causes the excitation of receptors spread over a much larger area An explanation on these lines has often been advanced to account for the relation between area and threshold intensity in the human eye[1] It is best indicated diagrammatically as in Fig 21, where the area and intensity of the illumination is shown by the shaded rectangles and the resulting excitation by the dotted line

[1] Fröhlich has assumed a spread of excitation round the stimulated area to account for the fact that the potential change in the eyeball is not limited to the illuminated area of the retina but it seems doubtful how far this result is due to the normal potential gradient which would be produced in the surrounding inert tissue of the eyeball.

We may regard the spread of the excitatory effect as due to some thing in the nature of a physical diffusion of an active substance over
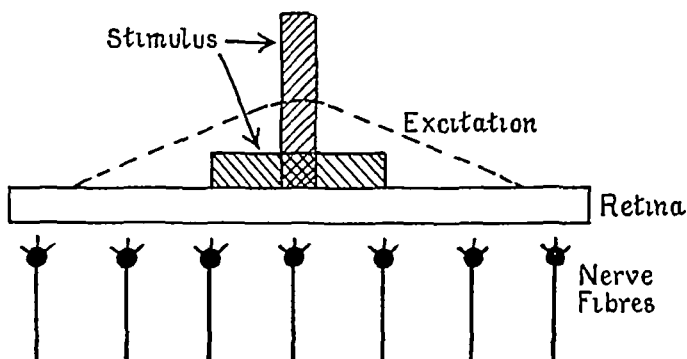

Fig 21 Diagram to show spread of excitation with restricted retinal image.
Intensity of illumination expressed by height of shaded rectangle

the surface of the retina, to a spread of current or to a radiation of nerve impulses in the synaptic networks If we are right in supposing that the retinal response indicates a process anterior to the excitation of the nervous structures the first two suggestions would be nearer the mark This must be left for further evidence, meanwhile we have to see whether such an explanation will really agree with the numerical data

The chief difficulty is to reconcile the idea of an excitation extending over a wide area with the relatively low impulse frequencies which are often encountered In the cutaneous and muscle receptors of the frog the frequency of the impulses in a single fibre seldom falls much lower than 5 per sec even with very weak excitation of the receptors, and is generally in the neighbourhood of 20–30 per sec It seems unlikely that the frequency in the optic nerve fibres covers a range which differs much from that of other sensory fibres and we may therefore take the figure of 5 per sec as about the lowest frequency which we can safely assume in a single fibre of the optic nerve Now the discharge frequency in the optic nerve with the smallest retinal images (10 to 70μ diam) is rarely below 50 per sec except at the beginning and end of the discharge when it is scarcely possible to speak of a definite frequency It is often nearer 100 per sec even with lights which are only just above threshold intensity Thus we may assume that as many as ten fibres are in action but it would be unsafe to assume a greater number Ten fibres of the optic nerve in the eel supply a disc of at least 25 mm diam on the retina (cf Appendix) and we may therefore suppose that

the excitation will spread over an area of this size however restricted the retinal image may be This amount of spread is large enough to be not inconsistent with our results though a larger spread might have suited them better The change in the latent period with the size of the image is greatest with areas up to 25 mm diam though it is still present with images up to 8 mm diam , and the dependence of the discharge on the total quantity of light is most clearly shown with retinal images below 5 mm though the frequency does not become directly proportional to the size of the image when this exceeds 5 mm The figures on which the argument is based are all such rough approximations that we can only say that a scheme like that in Fig 21 is not definitely ruled out The total number of impulses per sec is low, but the large retinal area supplied by a single optic nerve fibre is a sufficient reason.

There are many ways in which the scheme might account for the shorter latent period with greater quantities of light but the possibilities of varying rates of chemical change and of nervous interaction between different receptor elements or different neurones are so endless that further speculation would be of very little value at this stage

When we turn to the bearing of these results on the functional capabilities of the eye, we are met at once with the difficulty of explaining how the eye can signal the form of a stationary image of restricted size The simplest explanation is to suppose that in the eel it does not do so and indeed there is nothing in the behaviour of the animal to suggest that it does A spread of excitation of this kind would not interfere with an accurate signalling of the direction and rate of movement of a light or shadow, and this is all that we need postulate in such an eye But the relations between area and threshold intensity in the human eye are so clearly related to the intensity and area effects in the eel that it is hard to believe that there is any fundamental difference in the mechanism which responds to lights of low intensity in the human eye The development of a more specialised cone mechanism no doubt accounts for the fact that we can distinguish the size of very small objects On the other hand Pieron has shown that a spread of excitation seems to take place with red light which would affect only the cone mechanism, and Abney's results on area and threshold intensity were obtained with central as well as peripheral vision Thus the addition of the more critical cone mechanism brings its own problems with it

CONCLUSIONS

(1) Using a capillary electrometer and valve amplifier it is possible to record the action currents of impulses in the optic nerve of the eel (*Conger vulgaris*) and of the frog (*Rana temporaria*) when the retina is illuminated

(2) The action currents do not differ appreciably in time relations or in grouping from those in other sensory nerves, and their size is not affected by the strength of the stimulus (all-or-nothing relation)

(3) When the eye is illuminated, the discharge of impulses rises rapidly to a maximum frequency and then declines, at first rapidly and then more slowly   If the illumination has lasted a second or more, there is a renewed outburst of impulses when the light is turned off

(4) Owing to the rapid decline in the discharge with steady illumination, a movement of light and shadow in the visual field is a much more effective stimulus than a steady *pattern*

(5) When the retinal image does not exceed 1 mm diam the effects of an increase in the size of the image are the same as those of an increased intensity of illumination   Both the latent period and the maximum frequency of the discharge are determined approximately by the quantity of light in unit time (area × intensity), and the frequency is not directly proportional to the area of the retinal image   The relation between area and threshold intensity for the human eye is of the same kind

(6) When the retinal current and the optic nerve discharge are compared, it is found that the initial negative deflection (*a*) of the retinal response occurs at a fixed interval before the beginning of the optic nerve discharge   This "retinal-nerve" interval has an average value of 1 sec in the eel   Variations in the latent period of the optic nerve discharge are presumably due to variations in the latent period of the preceding retinal response

(7) The constant "retinal-nerve" interval is probably due to time lost in conduction through the synapses of the retina, and it is suggested that the retinal response (*a* deflection) is caused by the photochemical changes which precede the nervous excitation

(8) When the light is turned off, there is a constant interval between the beginning of the positive retinal response (*a'*) and the "off" discharge in the optic nerve   The "off" retinal-nerve interval is slightly shorter than the "on" interval, but the difference is not outside the range of experimental error

(9) The parallel effects of an increase of area and an increase of intensity can be explained by assuming a spread of the excitatory process into regions surrounding the illuminated area This agrees with the fact that it is impossible to confine the discharge to a single fibre of the optic nerve by limiting the size of the image

(10) Owing to the small number of fibres in the optic nerve of the eel and the large retinal area connected with one ganglion cell, a spread of the excitatory process is not ruled out by the relatively low frequency of the discharge

APPENDIX

THE ARRANGEMENT OF FIBRES IN THE OPTIC NERVE OF *CONGER VULGARIS*

In conger eels of 2–4 lb in weight the optic nerve appears as a thin, frail nerve, $1\frac{1}{2}$–2 cm long, and about 5 mm in diameter It is covered on its orbital half with a thick sheath Our first attempts to prepare good sections with osmic acid or Bielchowsky staining proved fruitless, but we were finally successful with a method suggested to us by the late Dr da Fano In this method the nerve is left in 5 p c osmic acid at room temperature for one day, and after changing the fluid is kept for two or three days in an incubator at 22–26° C After this the osmic acid is replaced with distilled water and the nerve kept in the incubator for another day or two, during which time the distilled water is changed twice or thrice Reduction of the osmic acid in the tissue continues in the tepid water Finally the nerve is transferred to an incubator of 37° C and kept there for some hours, dehydrated, cleared in xylol (1 hour) and embedded in paraffin This method left the structure of the nerve intact with no obvious signs of tears or shrinkage, the fibres were easily visible in sections about 2μ thick and could be counted from drawings with the camera lucida Good sections were, however, only obtained from the orbital part of the nerve

The chief points of interest revealed by the sections were the relative sizes and distribution of the fibres, and their small number   There are a few irregular strands of connective tissue, but no sign of the fibrous septa (primary and secondary septa of Leber) described in the optic nerve of man   Neither is there a central artery or vein   The most striking point is the way the fibres are grouped   there are large fibres in the middle of the nerve and small fibres round the periphery   The territory of the large fibres is distinct from that of the small, although in each part a few scattered fibres of the other size are to be seen   The difference in size of the fibres is not due to uneven shrinkage caused
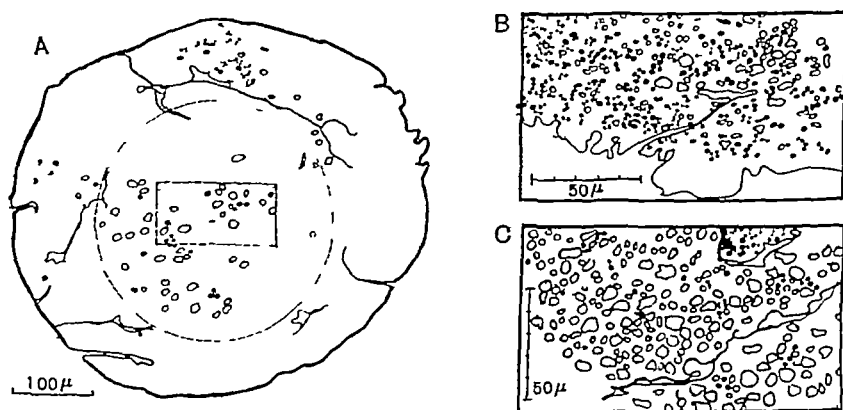

Fig 22   Camera lucida drawings of transverse sections of optic nerve of *Conger vulgaris*

    A. Low power   A few fibres drawn to give scale   Dotted circle shows approximate boundary between territories of large and small fibres   Rectangle gives area of Sections B and C

    B   High power   Fibres at periphery of nerve

    C   High power   Fibres at centre of nerve

by imperfect penetration of the osmic acid, since the diameter of the fresh nerve is not appreciably different from that of the nerve fixed and embedded in wax   Gudden(16) divided the optic nerve fibres of man into two classes, finer and coarser, and the same division can clearly be applied in the eel's optic nerve fibres   These vary in diameter from 1 to 8μ, and a rough division might be made by calling small fibres all those less than 3μ diameter, and large fibres those of diameter more than 3μ In a section with a total of 8000 nerve fibres ($\pm$ 1000) there were about 6000 of the small fibres and 2000 large ones   In four sections from different nerves the total number of fibres was 8000, 11,000,

10,000 and 9500   The appearance of about 80 sections from 5 nerves is substantially the same, so we may fix the average number of fibres in the optic nerve of conger at about 10,000 (cf 400,000 in man and in the cat)

Assuming that the nerve fibres are uniformly distributed on the retina, the total area of the retina divided by the total number of nerve fibres gives the minimum area which corresponds to one nerve fibre In all probability each fibre has connections with a wider area, for the retina has an extremely complicated structure of interconnected cells and there is no reason to suppose that the territories supplied by different nerve fibres do not overlap If we consider the retina as a hemisphere of diameter 6 4 mm , its area will be 64 sq mm Taking the number of nerve fibres as 10,000, the minimum area of the retina controlled by each fibre will be 6400 sq μ i e a square of side 80μ or circle of radius 45μ But the area may be even larger than this The optic nerve of mammals undoubtedly contains some efferent fibres If this is true for the eel, the number of sensory fibres is less than the total number of fibres in the nerve and the area corresponding to each on the retina is proportionally greater Gudden found in the cat and rabbit that the finer fibres in the optic nerve degenerated after destruction of the superior corpora quadrigemina, and considered these fibres to be efferent If the finer fibres in the optic nerve of the eel are efferent, the area of the retina corresponding to each of the remaining afferent fibres would be a circle of 100μ radius and area 03 sq mm

The structure of the retina of conger can be seen in sections stained with iron hæmatoxylin or Nissl's methylene blue and cut from eyes fixed in Flemming's solution   The cones can easily be distinguished from the rods by the size and position of their nuclei Both rods and cones appear to be evenly spread over the retina, but the rods outnumber the cones in the proportion of something like 20 to 1   In sections 5μ thick the ganglion cells in the eel's retina appear much more thinly scattered than the ganglion cells in similar sections of the frog's eye In the human fovea each cone is connected to a separate fibre of the optic nerve But in the retina of the eel the nuclei of cones visible in any one section always outnumber the ganglion cells, so that in most cases groups of rods or of cones, or of both, must be connected to each fibre of the optic nerve

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PROCEEDINGS

PHYSIOLOGICAL SOCIETY,

March 19, 1927

The effect of pH on the distribution of phosphorus between human red cells and potassium phosphate solutions By M. MAIZELS and A C HAMPSON *(Preliminary communication)*

Solutions of KOH, K_2HPO_4, KH_2PO_4, and H_3PO_4, of the same osmotic pressure, as determined cryoscopically, were mixed to give different hydrogen ion concentrations

Small quantities of hirudinised blood were placed in centrifuge tubes drawn out to a sealed capillary and spun for two hours at a speed of
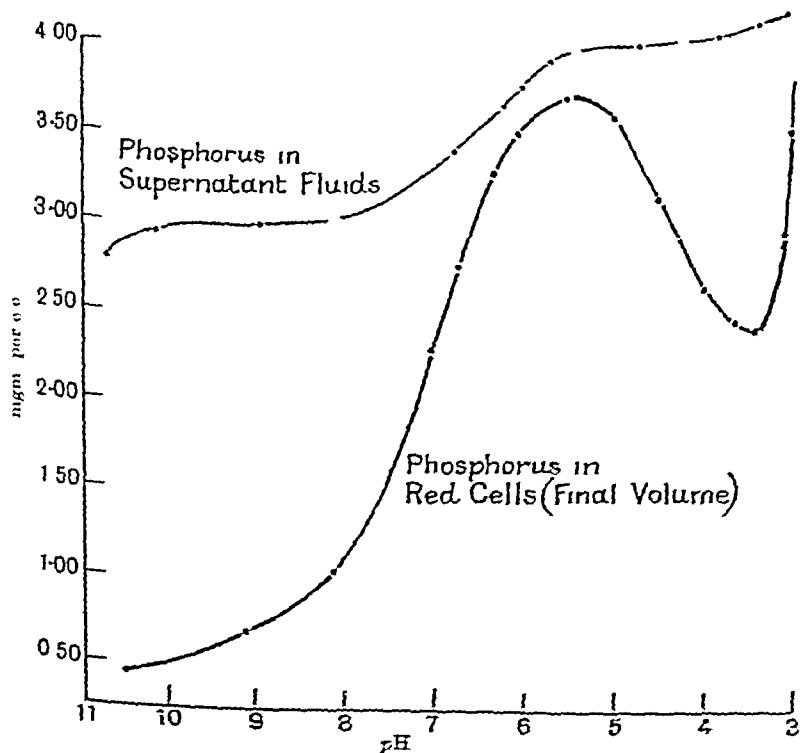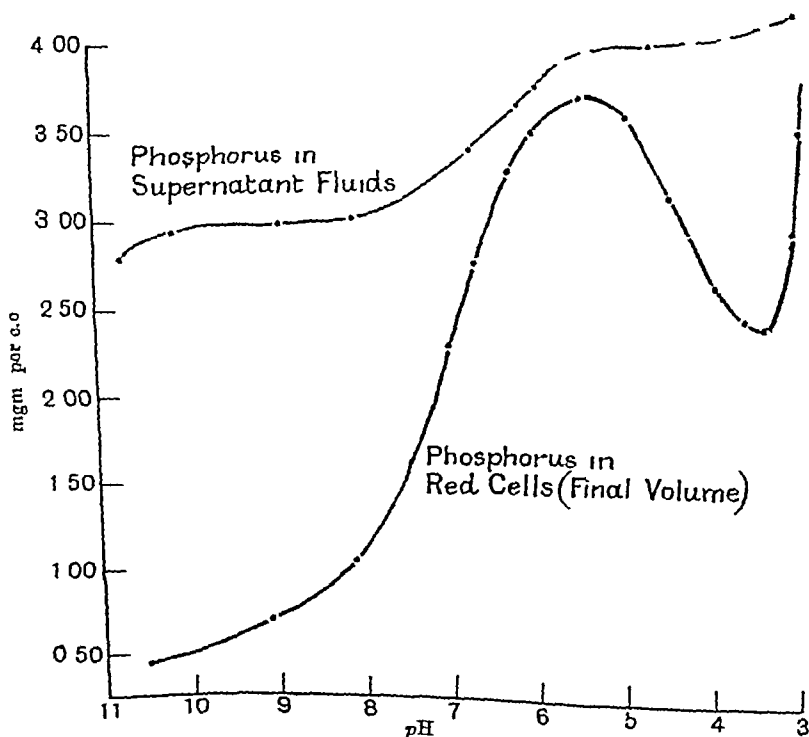
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Small quantities of hirudinised blood were placed in centrifuge tubes drawn out to a sealed capillary and spun for two hours at a speed of

a

three thousand revolutions per minute The volume of the cells was read and the supernatant plasma removed The cells were then mixed with 5 c c of the different phosphate solutions The suspensions were allowed to stand for one hour, and centrifuged for two hours more The volume of red cells was noted The inorganic phosphorus content of the red cells and supernatant fluid were determined by Briggs' method

From these data, the phosphorus content of the red cells per c c of final volume or original volume could be calculated

The following observations apply to red cells suspended in phosphate mixtures of $\Delta = 0\ 4225$ and concern the phosphorus content of the cells per c c of final volume and of the supernatant solution

(1) The phosphorus content at pH 8 1 is 1 mgm per c c of cells, as compared with 3 mgm per c c of fluid

(2) In solutions more alkaline than pH 8 1 the phosphorus content of the cells decreases

(3) In solutions more acid than pH 8 1 the phosphorus content rapidly increases till at pH 5 35 it is nearly equal to that of the supernatant fluid (3 75 mgm per c c of red cells as compared with 3 93 mgm per c c of fluid)

(4) In solutions more acid than pH 5 35 the phosphorus content falls, reaching a minimum at pH 3 6,—2 45 mgm per c c of cells, against 4 08 mgm per c c of fluid At this point slight hæmolysis occurs, and in still more acid solutions the phosphorus content rapidly increases till at pH 3 0 (approx) the phosphorus content of the cell practically equals that of the outside fluid

The concentration of phosphate ions can be determined from the pH of the liquid and the dissociation constants of phosphoric acid

Further work is in progress to determine the pH of the red cells under these conditions

Graded muscular contractions of natural form produced by electrical stimulation By GRACE BRISCOE (*Preliminary notice*)

The diaphragm and phrenics of cats have been used for experiment Owing to the bilateral character of this muscle, one-half can be paralysed and used for experiment, while the undisturbed half can be used as a model

Small hooks are attached to each dome on its abdominal surface and connected to simple crank levers The levers are unloaded The diaphragm

muscle is working under its natural thoracic load   One phrenic is dissected out and cut in the neck, the chest being intact, and stimulation applied to its peripheral end   An ordinary induction coil (usually coreless) is used, but the interruption is given by a Sherrington torsion wire key   With this key a very equable series of submaximal shocks can be delivered and make shocks can be eliminated   The rate of interruption (based on Gasser's(1) work on the action currents in dogs' phrenics) is 95 to 100 per second

A control tracing having been taken, the phrenic on one side is tied and cut   At once that half of the muscle ceases to contract, loses tone and rises higher than before nerve section   The action of the chest is increased and usually a passive inverse movement is imparted to the paralysed muscle   The secondary is placed as far as possible from the primary, and the short-circuiting key having been opened, it is pushed slowly towards the primary, until a small downward movement is observed on the paralysed side   This gives the threshold point   The observer watches the descent and ascent of the control side and pushes the secondary forwards and backwards in time with the control   By varying the pace and distance to which the secondary is advanced and withdrawn, a complete grading of contraction and relaxation can be obtained, so that a recognisable imitation of any type of respiration can be given   Fast types are naturally more difficult to follow

In natural respiration, the diaphragm is always in a state of postural contraction during the expiratory pause, though there may be considerable variations in the degree of postural tone   If the secondary is not withdrawn beyond the threshold point but is left during the expiratory pause at such a point that graded minute currents are passing into the nerve, then the muscle can be maintained in its natural expiratory posture   Thus a regular series of phasic contractions and postural contractions can be initiated and maintained in a paralysed muscle, and if the advance and withdrawal of the secondary are correctly timed, an almost exact reproduction of the natural movements and level of the muscle can be obtained
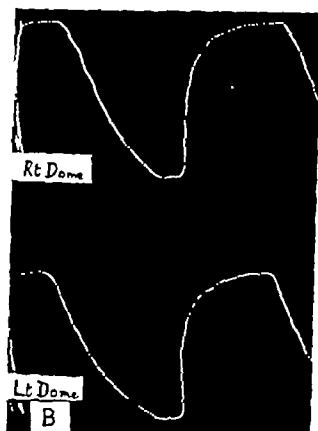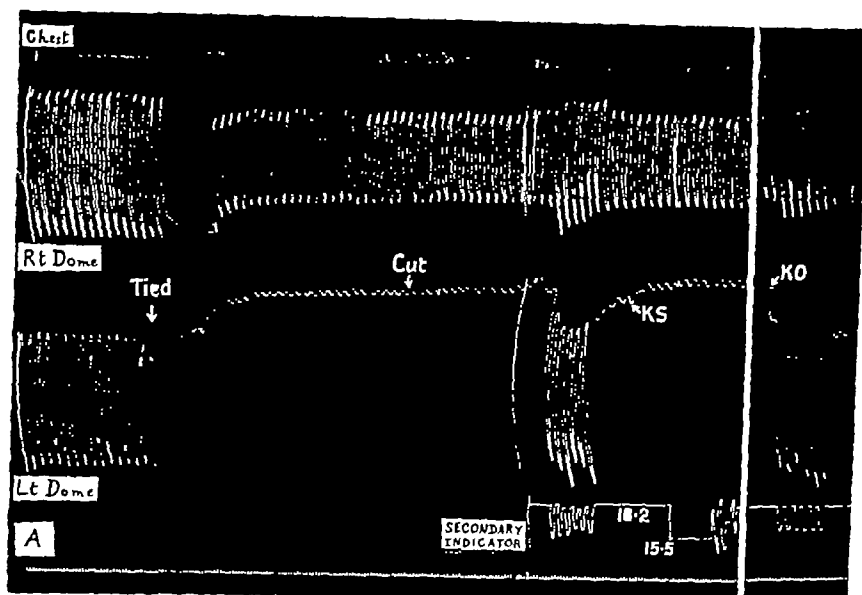
A. First part shows control tracing, both domes responding to a natural stimulus At
the first arrow the left phrenic is tied and later cut This results in loss of movement
and loss of tone The next group of six contractions are due to electrical stimuli
When the short-circuiting key is shut (*KS*) the muscle resumes its toneless level
Another group taken a few minutes later shows the expiratory posture at its natural
level. After the phasic contractions tone is maintained by minimal currents, the small
movements being passive. Time in secs

B Single contractions before nerve cut.

C. After nerve cut Left side paralysed. Time in secs

D Left side responding to an electrical stimulus

(1) *Gasser and Newcomer Amer. Journ. Phys.* 57 p 1

PROCEEDINGS

PHYSIOLOGICAL SOCIETY,

May 14, 1927

Rhythmical contractions of single heart muscle cells in tissue culture "in vitro" By Dr L. GAROFOLINI (presented by Prof GIULIO FANO)

We have known for a long time that fragments of heart muscle can show rhythmical contractions, and my own researches have demonstrated that this property is much more evident in the embryonic heart In tissue culture this rhythmical activity can last for a long time, as Carrel's and Burrows's first papers have shown, but we find very few observations in the literature on the contractions of single heart muscle cells. Researches by Engelmann in dying fragments of heart without culture and by Burrows, Roffo and Lewis on heart tissue culture give some accounts of it In my laboratory my assistant Dr Garofolini has found without changing the medium a peculiar behaviour in some heart tissue cultures from chick embryo at the 8th–9th day of development These cultures have grown very thin after the 6th–10th day of life *in vitro*, so that the single cells were easily delimited and the plexiform structure of the heart muscle very evident. There were formed some lacunar solutions of continuity in the original fragments crossed by thread-like myoblasts Of these cellular elements some contracted synergically with the rest of the fragment and others had individual rhythmical contractions variable from cell to cell from 40 to 110 pulsations a minute, but in every case very different from the rate of the general contractions of the fragment, which was much slower As the fragment was always dilating, some of these very thin myoblasts were broken, but the nucleated portion continued to beat at a rate analogous to that of the whole cell. The general rate of the fragment was very little influenced by changes in the temperature, which had appreciable effects on the autonomic cells These cells influenced one another reciprocally and those which contracted more strongly ruled the others to a certain extent For instance when an energically and regularly contracting cell came to rest another cell close by began immediately to contract regularly after having shown for a long time periodic and irregular beats

It is not easy to give an explanation of this. Certainly these cells show an automatic potentiality which can appear functionally also when the cells are not morphologically isolated from the syncytium   The automatic powers characteristic of earlier stages of embryonic development, which I have demonstrated in the third day of development especially in the cells lying at the venous end of the cardiac tube, are shown most evidently by the effect of the dedifferentiation in these cells during the life *in vitro*

We cannot forget that the products of katabolism have a certain influence on these cultures, but Olivo found in his researches on the dedifferentiation *in vitro* of the heart muscle that the increase in the automatism is accompanied by decrease in the excitability (exactly the opposite of what I found in the normal development of the organ) and we may therefore conclude that this influence should not be very great

### Culture "in vitro" of germinal epithelium from embryo chick
By Prof G FANO and Dr L GAROFOLINI

Many researches have been made on the culture *in vitro* of germ cells The studies of Goldschmidt on the fly testicle have stated that when the spermatogenesis has already begun *in vivo* it may continue *in vitro*, but in the testicle of adult of vertebrates such a differentiation does not occur, as we know from Champy   The same is the case for the female germinal epithelium of Mammah in (Champy) and for the human ovary (Zondek and Wolff)

We have made cultures from ovary and testis of the chick embryo from the 9th to the 19th day of development in pure homologous plasma or in plasma and embryo juice The cultures in plasma show a large outgrowth in the first two subcultures, but we have not succeeded in maintaining them living for more than six subcultures   The ovary cultures in plasma and embryo juice show a characteristic growth of the germinal epithelium, for the first 10 to 12 subcultures. When the plasma is laid in a very thin layer the outgrowth of the epithelium is very much like that of an endothelium, as shown by the liver endothelium in liver cultures (Lewis), only a little thicker and much more rapidly migrating   At the distal end of the migrating zone these syncitial layers which grow on the surface of the coverslip dissolve themselves into single cells charged with fat droplets and showing short and thick processes These cells become round, agglutinate themselves very often and finally degenerate   In a thicker layer of plasma the outgrowth of the

epithelium is somewhat different It grows from the culture in a sheet-like layer not very extended in surface and from this membrane it spreads out in the form of many strings, syncitial in appearance At the distal end also these strings show the dissolution into single cells and the agglutination of the latter as in the other type of migration

On account of the continuous degeneration of the distal cells the tissue, after a very remarkable outgrowth in the first 10 to 12 subcultures, begins to give a migration not so great as in the first days, with the character of the migration of fibroblasts Probably it is only when the germinal epithelium has exhausted its energy of growth that the fibro-blasts begin their migration We are still working on the testis of which we had very scanty material The enormous and rapid outgrowth in the first day of life of these cultures (particularly from embryos from the 8th to the 15th day of incubation) which no other epithelium shows, even in pure strain culture, can be understood in view of the fact that in those stages the gonads must still undergo much important modifica-tions, and therefore that during the embryonic period they can be con-sidered at a stage of development very inferior to that of all the other tissues and organs of the embryo
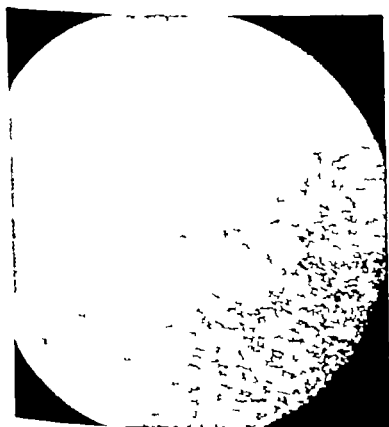
A   B

A. Ovary of embryo, 9th day
     4th transplant Endothelial appearance Isolated cells distally
     Obj 2 Koristka Fixed Maximow, stained iron haematoxylin.

B   Ovary of embryo, 15th day
     6th transplant Epithelium in laminae
     Obj Zeiss Apo 3 mm Fixed Maximow, stained iron haematoxylin

On functional recovery after severance of cutaneous nerves
By E SHARPEY-SCHAFER, Edinburgh

With the object of testing the relative rate of recovery of sensation in cutaneous nerves (a) completely severed with the knife or scissors and (b) severed by crushing, which would leave the nerve-sheaths intact but interrupt the fibres, I got a surgical friend, Mr Norman Dott, to sever the posterior division of the internal cutaneous nerve in each of my arms the nerve on the left side being cut across, that on the right side merely squeezed with fine artery forceps, tightly enough to destroy the continuity of the nerve-fibres The operations were done on October 11th, 1926, aseptically and under local anæsthesia the wounds were completely healed within a few days. In both cases—after the effect of the local anæsthesia had passed off—there was some pain referred to the area on the forearm supplied by the nerve this was no doubt the result of the traumatic injury which spreads upwards for a certain distance along the fibres. The severance of the nerve did not in either case produce complete loss of any kind of sensation in the part of the skin supplied by it This is accounted for by the fact that the nerve receives branches in its course from other cutaneous nerves, especially from the anterior division of the internal cutaneous besides which there is probably always some overlap from areas supplied by the adjacent nerves On both arms the area showed a slight but distinct diminution of sensibility for all forms of sensation The most striking effect was, however, a feeling to the touch which can best be described as "numbness", this affected the whole area supplied, which could by its means be easily mapped out In addition an œdematous condition was produced over the area. This is no doubt due to severance of vascular nerves it was much more noticeable and lasting on the left than on the right side. There was also for some time a painful tingling sensation, experienced also more on the left side than on the right This sensation was constant, but came to be ignored unless attention was specially directed to it it was probably due to the contraction of the cicatricial tissue which was forming at the site of the wound

Matters remained much the same for about six weeks After that time slight stimuli, such as a gentle prick, the pulling of a hair, or the pinching up of the skin, began to produce distinctly painful effects, at first only in certain parts of the numb areas, particularly near their upper end, but before long over the whole This hypersensitiveness to pain was more pronounced on the left side than on the right The pain

was not confined to the point stimulated but spread beyond it This has been noticed by all who have studied recovery after section of cutaneous nerves (Head and Rivers, Davies and Trotter, Boring) and is no doubt what Head and Rivers termed *protopathic sensation*— in contrast to *epicritic*—although why these names should be preferred to the old terms *general sensation* or *pain* and *special sensation* I do not understand. They have precisely the same meaning, for the sensation experienced under the above conditions is *pain* and nothing else It is probably caused by the growing parts of the nerve-fibres which subserve pain being hypersensitive, perhaps from the fact that they are either not at all, or only imperfectly, protected by the connective tissue sheaths which sooner or later form around all nerves, with the exception of the terminations of pain-nerves, which remain naked, as in the cornea[1]

It is now seven months after the operations described On the left side—that on which the nerve was cut across—the conditions remain much the same The feeling of numbness, the œdematous condition, the slightly lessened sensitiveness to touch, warmth and cold (the areas have always shown the specific "spots" for the various cutaneous sensations) the hypersensitiveness to pain, are all quite distinct Recovery of function is evidently very slow

But on the right side—that on which the nerve was crushed—the affected area began to show a diminution of extent within two months of the operation the diminution was very striking at three months At five months the part still affected occupied a very small patch Soon after, this had completely vanished and the whole area is now apparently normal

[1] We know nothing as to the modes of termination of "warmth' and "cold" nerves

PROCEEDINGS

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The migrations of chlorine ions   By R. K. CHRISTY, King's College, London

Experiments have been carried out on man which show that there is a definite alteration in the chloride content of whole blood during exercise, rebreathing of expired air and over-ventilation

The changes during exercise and the rebreathing of expired air are very similar—first there is a fall in the chloride content lasting for some time after the exercise and this is followed by a rise, this rise continues in some cases until the original value is exceeded   This may indicate an effort on the part of the body to make itself more capable of taking exercise in the future, in this connection it is interesting to note that the subject with the highest blood chloride was in much better training than the others

In over-ventilation the reverse changes take place, except that no over compensation occurs   The changes in the chloride content have not been found to be dependent on alterations in the hæmoglobin content of the blood.

It is suggested that an exchange of chloride ions occurs between the blood and the tissues similar to the Hamburger shift between the plasma and the red corpuscles and that the capacity of the blood to carry carbon dioxide is thereby augmented

The partition coefficient of ethyl iodide and its destruction by the tissues   By SAMSON WRIGHT and M. KREMER, Middlesex Hospital   (*Preliminary communication*)

The ethyl iodide method of Henderson and Haggard[1] for determining the circulation rate depends on two assumptions   (i) That the partition coefficient of ethyl iodide between alveolar air and arterial blood is approximately 2, (ii) That ethyl iodide is destroyed in its passage through the tissues, so that the venous blood contains less than 10 % of the concentration present in the arterial blood   Henderson

and Haggard determined the partition coefficient of ethyl iodide *in vitro* for water and blood, and *in vivo* in experiments on dogs Starr and Gamble(2) in experiments *in vitro* found the partition coefficient between air and water was 2 7, between air and blood *in vitro* the coefficient was found to be 7 6

Experiments on man were carried out by the present authors in which mixtures containing various concentrations of ethyl iodide were breathed for 10 to 20 minutes A series of maximal respirations were then taken from the outside air Ethyl iodide was still present in the alveolar air after 36 breaths, and after periods of 9 to 12 minutes The total amount of ethyl iodide given off in the expired air when breathing fresh air amounted to 1—2 % of the amount absorbed during the period that ethyl iodide was inhaled. The expired air was collected while ethyl iodide mixtures were breathed for 20 to 35 minutes. The ethyl iodide concentration was found to remain the same, or rise somewhat towards the end of the experiment

Attempts were made to determine the partition coefficient *in vivo* in experiments on cats and goats The animals breathed air containing 1—6 c.c. ethyl iodide in 250 L air to which CO was added In the case of the cats, samples of arterial blood and expired air were collected simultaneously and analysed The arterial blood was found to contain 2 3 to 7 8 times as much ethyl iodide as the expired air As the ethyl iodide concentration in the alveolar air is lower than that in the expired air, the true partition coefficient must be higher than the figures given. In the course of experiments lasting several hours the arterial ethyl iodide concentration was found to rise gradually, though the animal breathed steadily The venous blood contained 50—75 % of the concentrations present in arterial blood. Ethyl iodide was still present in the arterial blood if the animal breathed fresh air for 15 minutes In the goats, the alveolar air was collected by an electrical device (to be described later) and the partition coefficient was found to vary between 2 and 3 6

The dead space in the goat exceeds 80 c.c , as the tidal air in these experiments amounted to 120 c.c , it is highly probable that the alveolar air concentration obtained in these experiments are also too high

In these experiments ethyl iodide was estimated by the iodine pentoxide method This method was found to be reliable if a slow current of air is employed and the tubes are thoroughly seasoned

(1) Henderson and Haggard. Amer Journ. Physiol. 73 193   1925
(2) Starr and Gamble   Journ Biol Chem 71 509   1926

A relationship between body temperature and blood sugar in rabbits   By R D Lawrence

Some curious spontaneous rises in blood sugar accompanied by a lowering of body temperature have been observed in rabbits from time to time and examples of them are shown in the accompanying table They occurred on certain days without any apparent reason and were absent on other days in the same rabbits under standard experimental conditions The rabbits were on a constant diet, starved for 20 hours before the experiments   Similar phenomena have been recorded in certain pathological conditions, such as a prelethal rise in blood sugar with a low temperature in rabbits dying from diphtheria toxin[1] and from anaphylaxis[2], but the rises recorded here occurred in normal rabbits They were first observed after the injection of one unit of insulin, but were later found to occur spontaneously without any drug

Table I   *Blood sugar and temperature*

Rabbit	Day	0	½	1	2	3	4	5
				Hours of test.				
I	1	139 102° Insulin 1 unit	— —	75 102°	86 101 4°	412 97° (limp)	— —	— 101°
II	11	128 102 8° Insulin 1 unit	— —	71 103°	207 100 1° (limp)	129 101 7°	137 102 4°	—
IV	2	120 102 4° Insulin 1 unit	220 101°	158 101°	75 101 6°	51 102 4°	— —	— —
VI	1	130 103°	— —	135 102° Insulin 1 unit	247 102°	180 100 5°	108 100°	107 100 3°
	5	135 102°	204 100°	218 100 8°	177 100°	— —	123 102 9°	112 102°
	7	133 102 6° Ergotamine 0 5 mg	116 101 4°	114 101 4°	113 101 8°	102 102 3°	— —	— —
	9	131 103°	135 102°	135 102° Adrenaline 0 5 mg	248 102 5°	420 102 2°	347 101 8°	307 102°
	20	110 102 8°	108 103°	110 102 4°	110 102 4°	— —	— —	— —
VII	5	140 101 2°	117 101 8°	— —	118 101 9°	117 101 6°	— —	— —

Remarks Rabbits I, II and IV gave normal insulin reactions on all the other days they were tested and did not seem especially excitable Rabbit VI was very excitable and gave a rise of blood sugar on every day he was tested until ergotamine was given It seems likely that ergotamine controlled the rise of blood sugar, although it is impossible to be sure that it would have occurred in its absence Adrenaline produced an exceptionally high blood sugar in this rabbit without, however, any fall in temperature Rabbit VII shows a much *commoner* condition of blood sugar instability observed in many rabbits in which the initial blood sugar is raised but falls to a normal stable figure later

Discussion It is impossible to investigate the causes of these unexpected rises experimentally because one never knows when they are going to occur Marked rises have occurred only in seven out of about 100 rabbits used for such tests and in some of them occurred only on the first day of testing when they were excitable and unaccustomed to handling The obvious suggestion is that they depend on an increased secretion of adrenaline, and this is supported indirectly by an observation of Geiger(3) that the hyperglycæmia which invariably occurs in rabbits when their temperature is lowered by artificial cooling, is prevented by bilateral splanchnectomy The prelethal rise in blood sugar of the collapsed rabbit is also prevented by ergotamine which paralyses the adrenaline mechanism. It is suggested, therefore, that these curious rises of blood sugar are adrenaline reactions produced sometimes by mere excitement, sometimes as a reaction to the fall in blood sugar caused by insulin (a well-known phenomenon) and sometimes as a reaction to a collapse of no obvious origin In the collapsed cases the low temperature would seem to be primary to the high blood sugar, because the hyperglycæmia produced by an injection of adrenaline is not accompanied by a low temperature It is not likely that the high blood sugar is due to the failure to utilise sugar in conditions of collapse, because then the hyperglycæmia produced by cooling would not be abolished by splanchnectomy

A point of practical importance arises, that, before testing the effect of any substance on the blood sugar of rabbits, it is important to ensure that the blood sugar is stable by doing more than one preliminary test

(1) Lawren ‹ *P H* ‹ _l Puckley, Q B British Journ. of Exper Path. 8 58 1927
(2) O Neill ‹· ‹l J › r _ Am r Med. Assoc. 74 1102. 1925
(3) Geiger E. ‹~ v f *Expr* Path. u. Pharmak. 121 67 1927